# STATOCYST INTERNEURONES IN THE OPTIC TRACT OF A PORTUNID CRAB, *SCYLLA SERRATA*

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*I declare that all original work presented in this thesis is my own.*



*Barry O'Brien*

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The responses to rotation of a series of interneurons in the optic tract of a portunid crab have been studied.

A diversity of units has been found. Two have tonic responses to maintained tilt. Three have phasic non-directional responses to rotation. The remaining ten show phasic directional responses to rotation.

The orientations showing the most directional responses include most possible orientations, although a higher proportion respond best to pure roll.

In all cases the peak response leads the peak position during sinusoidal oscillation.

From a consideration of many properties it is concluded that most units have input from the statolith. This includes many purely phasic units.

Most of the interneurons are bi- or multimodal. The significance of these properties in determining the response to rotation is discussed.

Several possible functions for vestibular input to the optic lobes are proposed.

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# CHAPTER I

# INTRODUCTION

The brachyuran statocyst is probably the most sophisticated arthropod spatial equilibrium organ. Only the cephalopods among the other invertebrates have a similarly complex organ (for a comparative study see Markl 1974).

The statocyst is differentiated into two more or less discrete canals, one horizontal and one vertical (Hensen 1863). The canals contain two sorts of sensory hairs, the thread hairs and the free hook hairs, both of which have been shown to be sensitive to angular accelerations (Dijkgraaf 1955). Situated on the wall of the lower portion of the vertical canal is a statolith, an aggregation of sand particles, mounted on two incomplete rings of hook hairs known as the statolith hairs. Since its discovery in crabs by Dijkgraaf (1956) the statolith has been considered to monitor tonic displacement of the animal from the equilibrium position. For details of the structure of the crab statocyst see Sandeman and Okajima (1972a) and Dunn (1975).

The earlier studies of statocyst function have concentrated on behavioural effects of selective elimination of the input from the various hair types. From studying the compensatory eye movements Dijkgraaf (1956) concluded that the canal hairs, particularly the thread hairs, were responsible for the vestibular control of eye movements elicited by continuous rotations, the statolith having a regulatory function and controlling the eye position in response to maintained tilt. This analysis of the role of the thread hairs has been confirmed and extended by Sandeman and Okajima (1972, 1973a & b). The statolith is still regarded as a monitor of maintained tilt in spatial equilibrium, the

few recorded primary sensory responses from crabs showing a very slowly adapting response to maintained tilt (Sandeman and Okajima 1972, Dunn 1975). The suggested role of the statocyst in vibration reception will not be considered here.

Although it has long been known that it is possible to record purely phasic responses to movement from vertebrate otoliths (Lowenstein and Roberts 1949) similar responses have never been obtained from the crab statolith, which is considered an analogous organ. However it has recently been demonstrated that both phasic and phasic/tonic (slowly adapting) responses can be recorded from directly manipulated crab statolith hairs (Janse and Sandeman, in preparation).

Although statocyst-induced eye movements and statocyst sensory responses are now known in some detail the intervening steps are in most cases not well known. The eye movements induced by stimulation of the horizontal canals are the only exception to this. Here a very direct connexion between the sensory and motor neurons has been shown, with a suggestion that no interneurons need be involved (Silvey and Sandeman 1976b).

The only extensive analyses of statocyst responses in the crab central nervous system have been by Fraser (1974, 1975a & b) and Fraser and Sandeman (1975) who showed that the rotational sensitivity of several units in the circumoesophageal connectives clearly derived from the thread hairs. Free hook hair input to one interneuron was also demonstrated. No clear function could definitely be ascribed to this unit although its response coincided with certain behaviours. No statolith responses have been reported from the circumoesophageal connectives, despite the importance of the lith in postural control (Dijkgraaf 1956).

The only direct recordings of statocyst input to crab optic lobe are based on responses to vibration and manual rotation of the antennule base (Bush *et al.* 1964) a stimula-

tion which might also excite antennule proprioceptors. Ablation experiments have led Wiersma and various co-workers to infer an inhibitory statolith input to the optic lobes in both crabs and crayfish (best summarised in Wiersma 1975). The statocyst input is thought to inhibit part of the visual field of visual units known as "space constant fibres". These were considered to have primary sensory input from the statolith, although this has not yet been demonstrated.

This brief summary underlines the paucity of knowledge of the central nervous processing of statocyst information, particularly of the statolith hair responses.

The decapod optic lobes have been shown to be of importance in more than just visual processing. The medulla terminalis, perhaps not a true optic ganglion (Maynard and Yager 1968) has been shown to play a role in olfactory integration (Maynard and Yager 1968) and in postural control (Hazlett 1971) although neither study has shown the extent of the processing occurring at this site.

Using the approach of central nervous system recording this study examines the ability of the crab to discriminate the direction of rotation in the vertical planes and to detect maintained position when displaced from the horizontal. The rotational stimulus is the oscillation of a pendulum released from a constant displacement and oscillating at a constant frequency. By recording from the tract connecting the optic lobes to the brain it was hoped also to find units mediating the space constant effect.

# CHAPTER II

# METHODS AND TECHNIQUES

*YAW, ROLL AND PITCH*

Throughout the text the terms yaw, roll and pitch will be used to describe rotations in particular planes. The three planes are at right angles to one another and correspond to the horizontal (yaw) vertical transverse (roll) and vertical longitudinal (pitch) planes (Fig. 1). The terms "head-up" (hu) and "head-down" (hd) will be used to indicate the sense of movement in the pitch plane. Roll will be described as "right-side-up" (rsu) "left-side-down" (lsd) or "clockwise" (cw) in one sense and "right-side-down" (rsd) "left-side-up" (lsu) or "counterclockwise" (ccw) in the other. The choice of term for a particular direction of movement will depend on which statocyst appears to be involved. "Clockwise" (to the right) and "counterclockwise" will also be used to describe rotations in the yaw plane. The common terms for yaw, ipsi- and contralateral, are confusing when units from both statocysts are found in each lobe, and where it is unclear which statocyst is being recorded from.

*RELATIONSHIP BETWEEN POSITION, VELOCITY AND ACCELERATION*

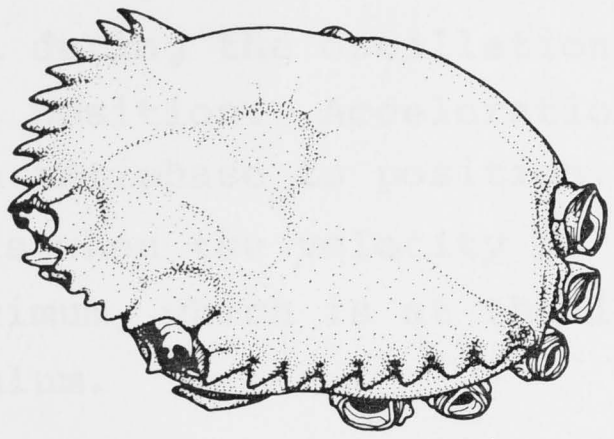
On both the instantaneous frequency plots and the oscilloscope records the lower trace is a monitor of pendulum position (e.g. Fig. 19). Because of this the phase relationship of a response will be expressed as leading or lagging position by a stated amount. In addition the peak of the response will sometimes be related to the peaks of

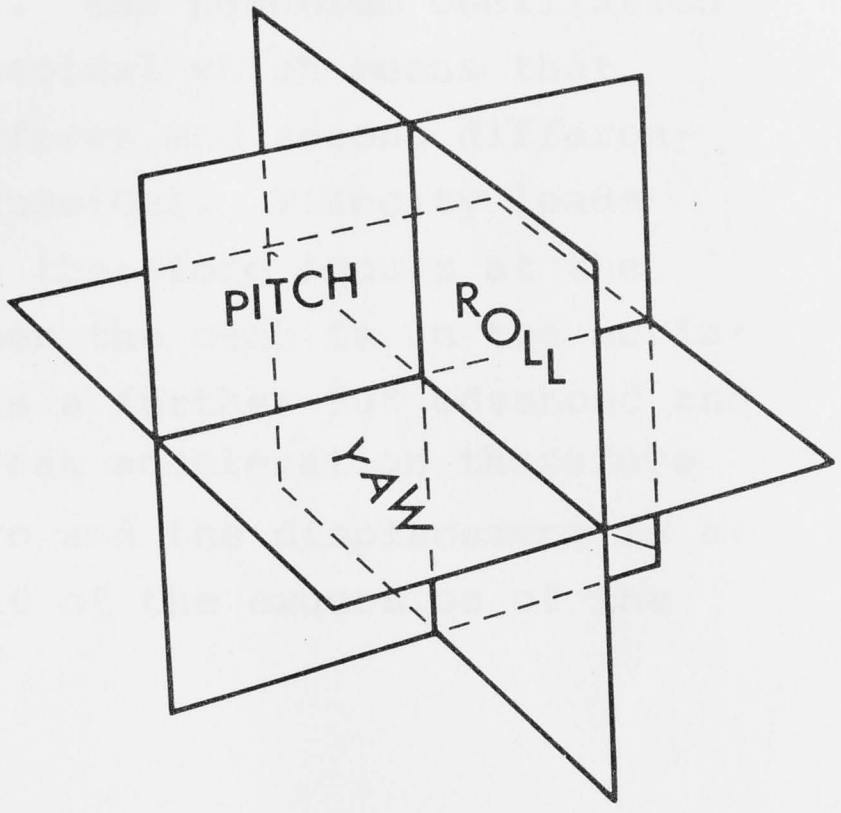
*Figure 1*

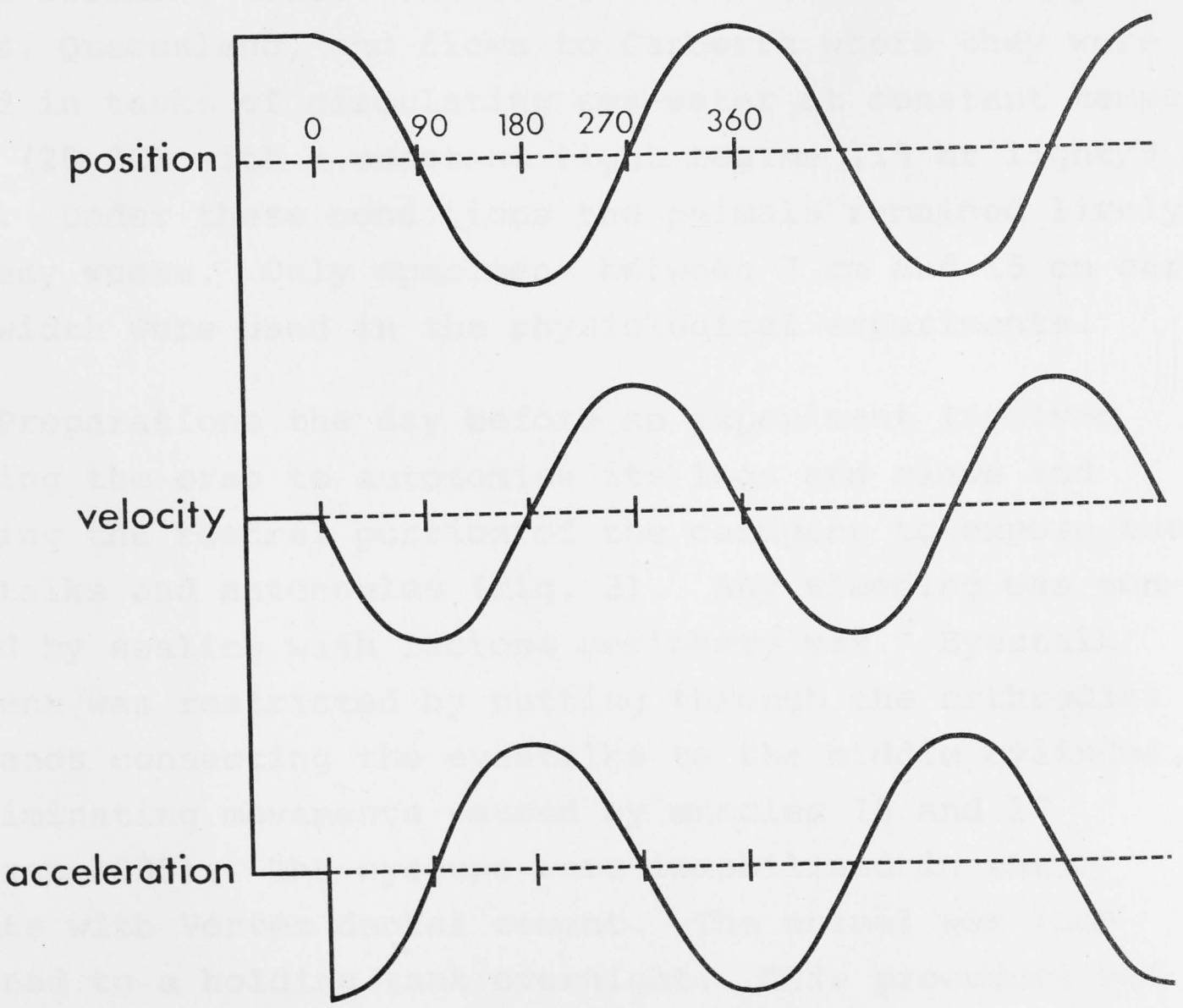
The relationship of the three principal planes of movement used in the experiments. See text for details.

*Figure 2*

The relationship between position, velocity and acceleration of the pendulum. The oscillation is calibrated in degrees, one oscillation comprising 360 degrees.




PITCH
ROLL
YAW


0
90
180
270
360
position
velocity
acceleration

velocity and acceleration. The relationship between these parameters is shown in Figure 2. The pendulum oscillation is assumed to be perfectly sinusoidal which means that velocity and acceleration, the first and second differentials of position, are also sinusoidal. Velocity leads position by 90°. Peak velocity therefore occurs at the point during the oscillation when the crab is in the horizontal position. Acceleration is a further 90° advanced and is in antiphase to position. Peak acceleration therefore occurs when the velocity is zero and the displacement is at a maximum, which is at the limit of the excursion of the pendulum.

*PREPARATION OF THE ANIMAL FOR EXPERIMENTATION*

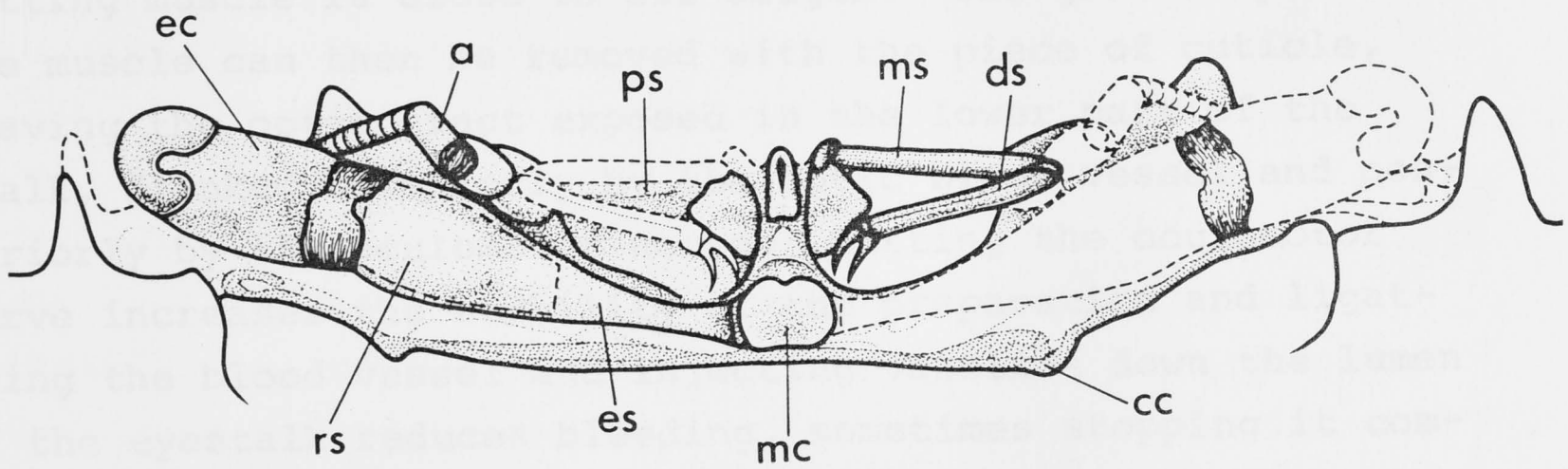
The species used in this study was *Scylla serrata* (Forskal) (Sect. Brachyura, Fam. Portunidae) a large but active swimming crab. The crabs were collected weekly in Cairns, Queensland, and flown to Canberra where they were stored in tanks of circulating sea-water at constant temperature (20 °C) with a constant light regime (15 hr light/9 hr dark). Under these conditions the animals remained lively for many weeks. Only specimens between 7 cm and 15 cm carapace width were used in the physiological experiments.

Preparations the day before an experiment involved inducing the crab to autotomise its legs and claws and removing the rostral portion of the carapace to expose the eye stalks and antennules (Fig. 3). Any bleeding was contained by sealing with Lactona periphery wax. Eyestalk movement was restricted by cutting through the arthrodial membranes connecting the eyestalks to the middle cylinder, so eliminating movements caused by muscles 15 and 17 (Cochran 1935). The eyecups were immobilised in their sockets with Vertex dental cement. The animal was then returned to a holding tank overnight. This procedure was intended to reduce the trauma to the animal immediately

*Figure 3*

A dorsal view of the anterior part of a crab prepared for experimentation.

Abbreviations: a – antenna; cc – cut edge of the carapace where the rostral portion has been removed; ds – distal segment of the antennule; ec – eye cup; es – eye stalk; mc – middle cylinder; ms – medial segment of the antennule; ps – proximal segment of the antennule, which contains the statocyst; rs – section of eye stalk cuticle (bounded by dotted lines) which is removed to expose the optic tract.


ec
a
ps
ms
ds
rs
es
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cc

prior to the experiment.

For the experiment a crab was mounted in the trough (Fig. 4) with the carapace margin horizontal. This provided a convenient reference position although it corresponds to about 15° head down from the normal bodily orientation of the resting animal (Dunn 1975).

The optic tract is an axonal tract running between the optic ganglia, situated in the eye cups and the supra-oesophageal ganglion, or brain. It was exposed by cutting the eyestalk cuticle in the region shown in Figure 3 and cutting muscle 18 close to its origin. The greater part of the muscle can then be removed with the piece of cuticle, leaving the optic tract exposed in the lower part of the stalk, flanked anteriorly by the optic blood vessel and posteriorly by the oculomotor nerve. Cutting the oculomotor nerve increases the stability of the preparation and ligaturing the blood vessel and injecting vaseline down the lumen of the eyestalk reduces bleeding, sometimes stopping it completely. The most stable recordings were obtained from the desheathed unsupported tract. Recordings were not restricted to either tract but only one was recorded from each animal.

*ELECTROPHYSIOLOGICAL RECORDING TECHNIQUES*

Various recording techniques were used, including hooks, suction electrodes and insulated pins, but the most profitable electrode was made by breaking the tip of a conventional micropipette to an internal diameter of 10-30 μ and filling with ringer. This type often gave large signals, even from units with statocyst input, although these were not among the largest units recorded.

Movement artifacts and mains interference, resulting from using a long lead from the moving apparatus to the pre-

amplifier, were overcome by feeding the signal first to a low impedance preamplifier (Tektronix Type 122) and then to an AC-coupled Grass P16B microelectrode amplifier (filter settings: AC out TC – spike filter; Rise Time – 300 μsec) before being displayed on a conventional cathode ray oscilloscope. Without this combination of impedance matching and filtering satisfactory recordings could not be obtained.

*THE STIMULATING APPARATUS*

The rotational stimulus was provided by an oscillating pendulum. Figures 4 and 5 show two views of the pendulum. In the plan view a crab in unprepared state is held in the plexiglass trough (t) by two wires attached to bolts in the base of the trough. The trough is screwed to the revolving plate (rp) which is free to revolve with respect to the baseplate (bp) unless held in position by the three clamps (c). The micromanipulator (m) and electrode holder (eh) are the only other structures mounted on the revolving plate. The amplifier lead (al) passes from its attachment to the first preamplifier (Tektronix 122) which is mounted directly above the apparatus.

The knife-edges (k) are attached to the base plate (bp) and rest on bearing surfaces mounted on the frame (f) which rests on the steady bench. This allows the baseplate and all structures attached to it to oscillate with respect to the frame about the axis set by the knife edges. Although the revolving plate is free to rotate (in the plane of the page in Fig. 4) the baseplate does not revolve in any direction other than the one plane allowed by the knife edges. With the crab in the position figured this would constitute a roll oscillation.

The position marker (pm) is a phototransistor used as a photovoltaic cell to give a positive voltage at the base, negative at the collector. The emitter was not connected.

A light source and the phototransistor were positioned on either side of one knife-edge so that when the pendulum oscillated the knife-edge entered the light beam, causing a drop in voltage. The voltage did not vary perfectly sinusoidally, as is shown on the traces, but it allowed accurate determination of phase relationships and had absolutely no damping effect.

Another view of the apparatus is shown in Figure 5. The revolving plate is shown clamped to a locking plate (lp) which is rigidly attached to the base plate and cannot revolve. The structure so far described is counter-balanced by a weight (w) which is rigidly attached by a shaft to the centre of the base plate. The knife edges are shown resting on the bearing surfaces on the frame (dotted outline). The whole structure in solid outline is free to oscillate in the plane of the page. The crab in the position figured would experience a rolling movement.

In all rotation experiments the pendulum was deflected 10°, to the left in the diagram, and released from this position either by hand or with an electromagnet. The crab in the orientation figured would therefore be released from a 10° right-side-up position, which corresponds to the 0°/360° orientation in the polar plots.

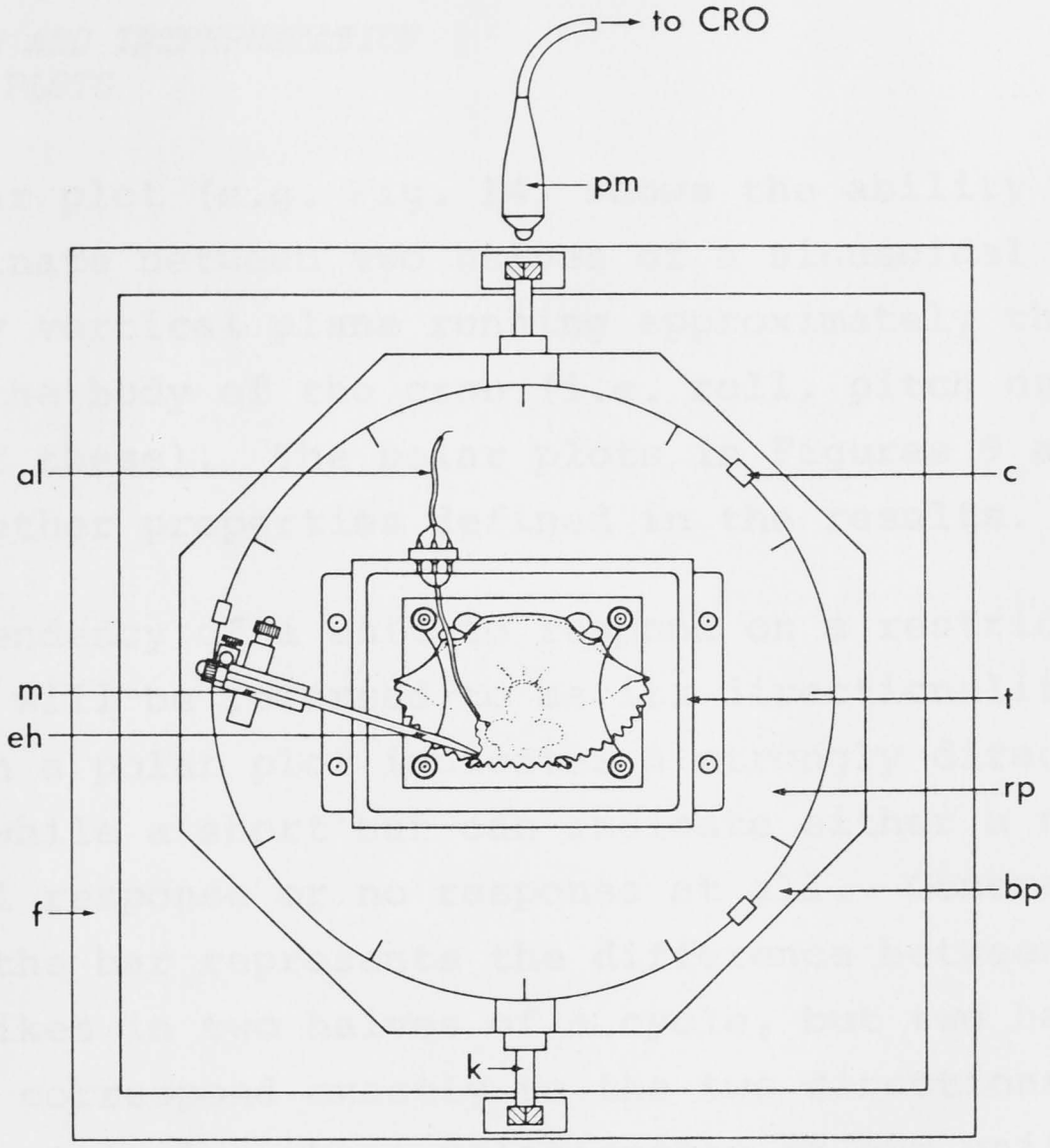
The pendulum oscillated at a frequency of 0.56 Hz. Although subject to some damping from wires and air friction, an initial 20° peak-to-peak oscillation still exceeded 18° ptp after 8 oscillations, by which time all polar data had been obtained. All polar plots, unless otherwise stated, were determined on oscillations with an initial displacement of 10° from the vertical, giving a peak angular velocity of $35°.sec^{-1}$ and a peak angular acceleration of $124°.sec^{-2}$.

*Figure 4*

Plan view of oscillating apparatus. See text for details.

*Figure 5*

Elevation of oscillating apparatus. See text for details.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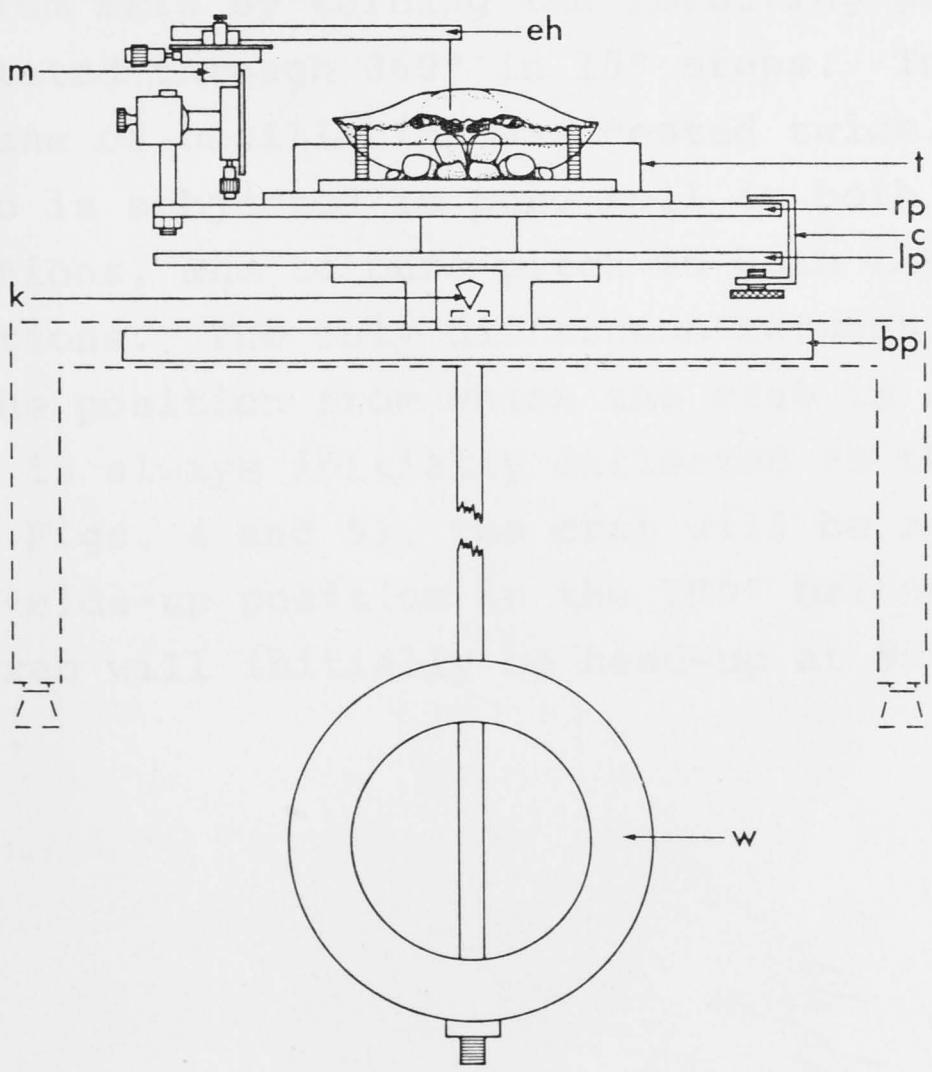
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*DETERMINATION AND INTERPRETATION OF THE POLAR PLOTS*

A polar plot (e.g. Fig. 14) shows the ability of a unit to discriminate between two halves of a sinusoidal oscillation in any vertical plane running approximately through the centre of the body of the crab (i.e. roll, pitch or any combination of these). The polar plots in Figures 9 and 12 represent other properties defined in the results.

The tendency of a unit to respond on a restricted part of a cycle will be referred to as its directionality. A long bar on a polar plot indicates a strongly directional response, while a short bar can indicate either a non-directional response or no response at all. Generally the length of the bar represents the difference between the numbers of spikes in two halves of a cycle, but two halves do not always correspond exactly to the two directions of movement, the particular phase relationship of the unit determining the dividing point.

The plots were made by keeping the axis of rotation of the pendulum constant (fixed by the knife edges) and progressively altering the orientation of the crab with respect to the pendulum axis by turning the revolving plate. The plate was rotated through 360° in 15° steps. This means that each plane of oscillation was tested twice. For example, the crab is subjected to pure roll in both the 180° and 360° orientations, and to pure pitch in both the 90° and 270° orientations. The only difference between these orientations is the position from which the crab is released. As the pendulum is always initially deflected to the same side (the left in Figs. 4 and 5), the crab will be released from the 10° left-side-up position in the 180° orientation. Similarly the crab will initially be head-up at 90° and head-down at 270°.

*THE RESPONSE/STIMULUS PHASE RELATIONSHIP*

Two different techniques have been used to determine the phase relationship between the response and the stimulus. The instantaneous frequency has been determined by measuring, with the aid of a microscope, the interspike interval from filmed recordings and the derived rate plotted against pendulum displacement. This procedure is responsible for the stratification apparent in the higher frequencies of the instantaneous frequency plots. The position of the peak discharge has been determined as the point of intersection of lines drawn through the regions of linear increase in frequency. The phase angle is then taken as the difference between this peak and the peak pendulum displacement.

An alternative method involved measuring the phase angle between the median spike in a burst and the peak displacement. This technique, which is relatively insensitive to a low-frequency tailing off of the discharge, was used only when the discharge was more or less symmetrical. The two approaches gave very similar results, with the median spike method often being used when the frequency peak was difficult to determine precisely.

*POSITIONS OF APPARENT PHASE CHANGE*

Consider a unit firing to head-down pitch. Obviously when in the 90° orientation (initially head-up) it will fire in the first half of each oscillation, but in the 270° orientation (initially head-down) it will fire during the second half. This is illustrated in Figures 39 and 40, where there appears to be a phase shift of 180°. The orientations in which this shift occurs should be at right angles to the orientations showing maximum directionality. They will be referred to as the "positions of apparent phase change", and marked on the polar plots with arrows.

*THE EFFECT OF OFF-AXIS ROTATION*

It is apparent in Figure 5 that any rotation imposed on the crab will be about an axis which does not pass through either statocyst. Off-axis rotations include both translational and rotational components. On the pendulum the linear forces will vary sinusoidally in phase with the angular forces, the relative magnitude of each being determined by the displacement of the receptor from the axis of rotation. Any structure sensitive to shearing forces would be stimulated by the translational component. All statocyst hairs are in this category (Janse and Sandeman, in preparation), although the effect on the thread hairs is likely to be very small. Rotational and translational movements are not readily distinguished between by a hair responding to deflection unless the mechanics of the sense organ first make the discrimination. For example, a thread hair responds well to displacement but the architecture of a canal does not allow brief translational movements to be detected by thread hairs. Any lith organ will respond well to both types of movement within a frequency range set by the mechanics of the particular system.

With the exception of pitch about an axis running through both statocysts, a very unlikely situation in the free-living animal, all rotations experienced by a crab would have an off-axis component in at least one statocyst and probably in both. Thus the types of movement experienced by the free-living animal are all represented in the stimulus the experimental animal receives, albeit in a simplified and stereotyped combination.

*THE ORIENTATION OF THE STATOCYST CANALS*

The orientation of the statocyst canals is shown in Figure 6, modified from Sandeman (1975). The essential feature to note is that the two vertical canals are oriented

approximately at 90° to each other and are at 45° to both the longitudinal and transverse axes of the body. The response to rotation of any unit in the canals will depend on the magnitude of the rotational component in the plane of the canal. For a receptor with a linear discharge frequency/displacement curve the response can be expressed by the formula

$$R' = R_0 \cos \theta$$

where R' is the response in a given orientation

$R_0$ is the response tc rotation in the plane of the canal

and $\theta$ is the angle between the plane of rotation and the plane of the canal.

This is shown graphically in the theoretical plots of vertical canal response in Figures 7 and 8. As the plane of the left canal is oriented along the 135-315° axis rotation in this plane gives a maximal response ($\theta = 0°$, $\cos \theta = 1$). When the animal is rotated in the vertical plane set by the 45°-225° line, there is no component of rotation in the plane of the vertical canal, and there is no response ($\theta = 90°$, $\cos \theta = 0$).

The right canal responds in a similar manner with the optimum response being on the 45°-225° axis.

These patterns have been confirmed by Fraser and Sandeman (1975) who have obtained very similar plots from an interneuron with thread hair input.

*Figure 6*

A dorso-posterior view of the arrangement of the semi-circular canals in *Scylla serrata*. The planes of the vertical canals are at 45° to the longitudinal and transverse axes of the animal. The axis labelled "horizontal axis" is actually the horizontal transverse axis. Modified from Sandeman (1975).

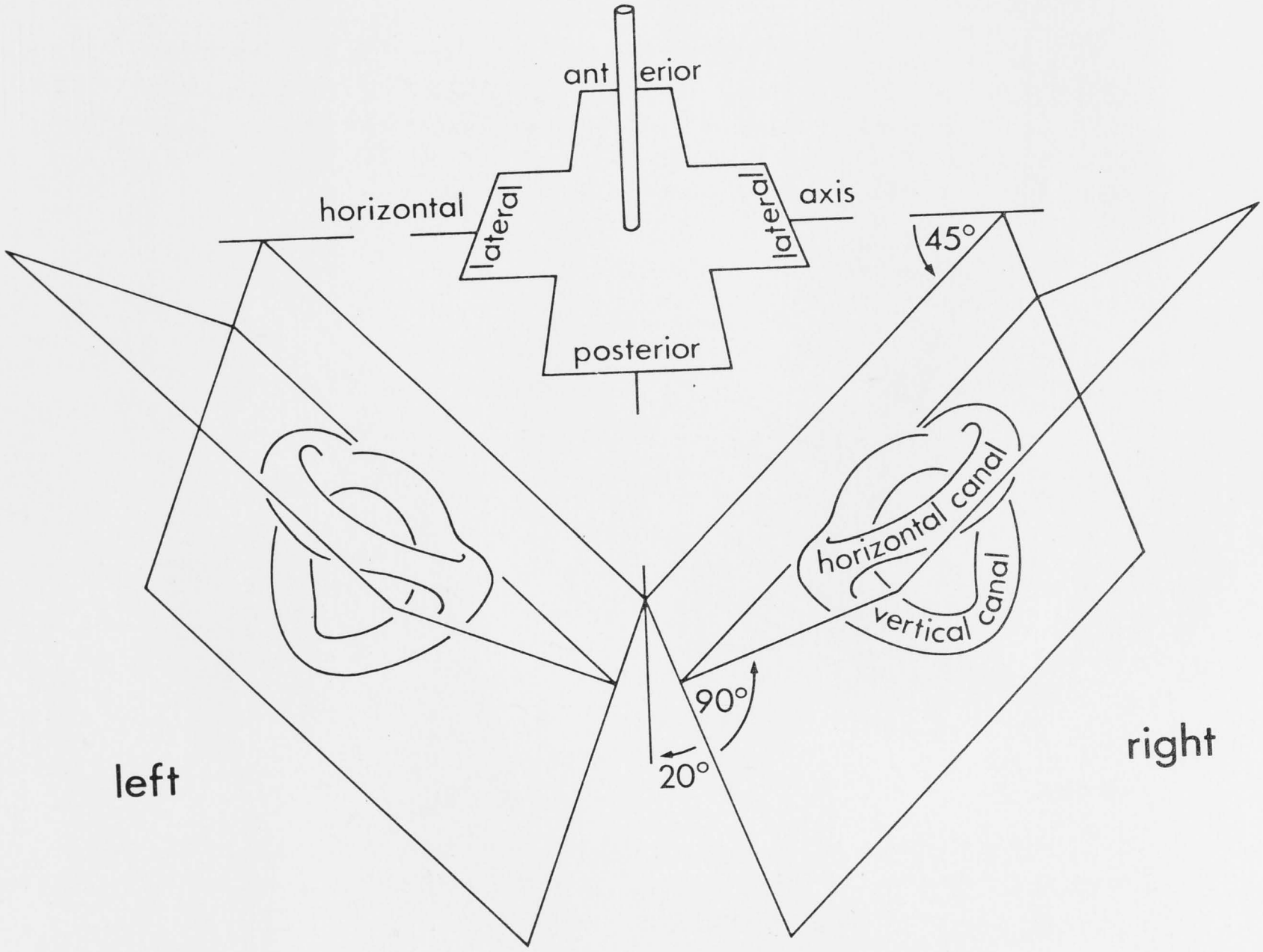
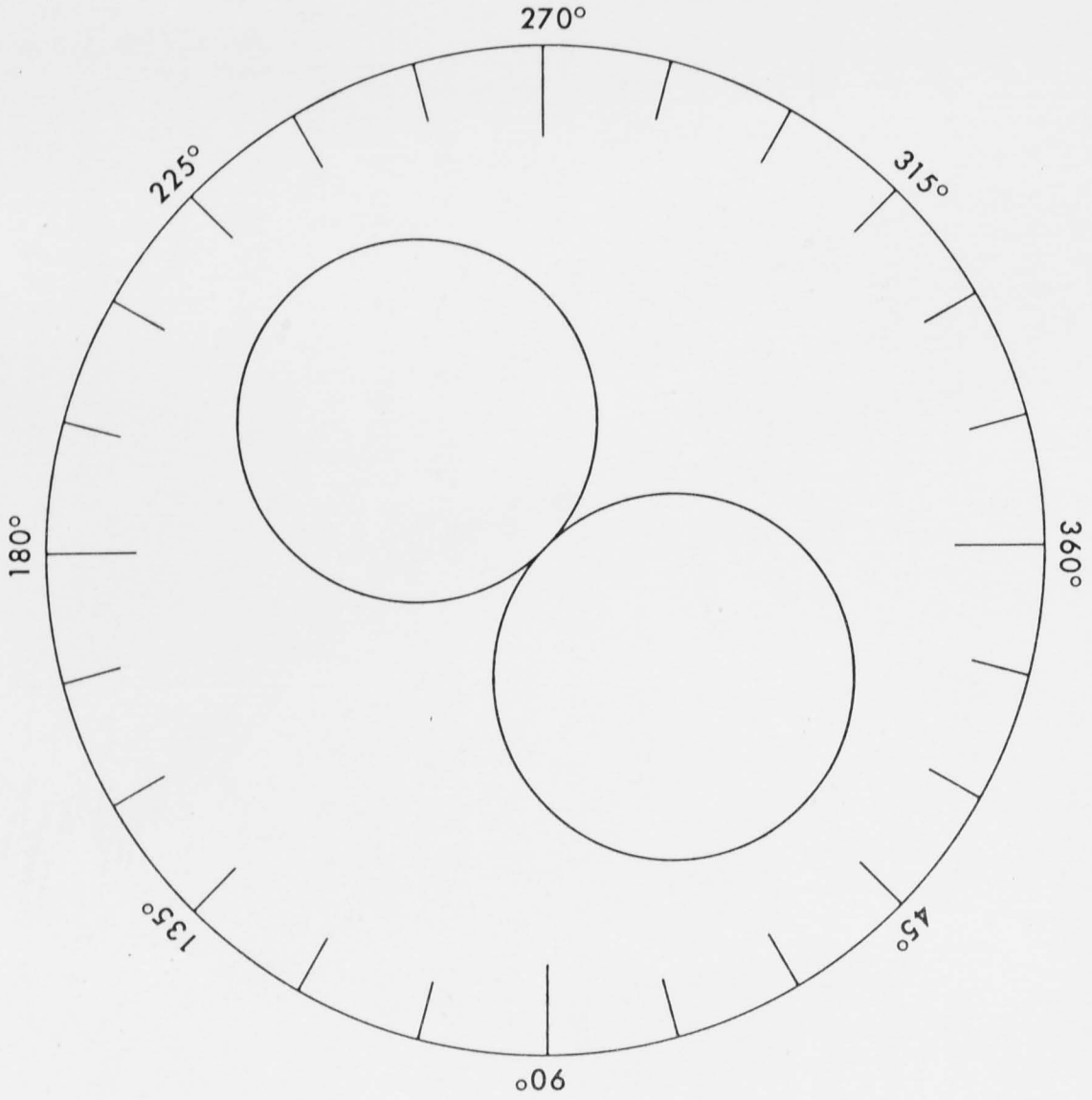

anterior
horizontal
lateral
lateral
axis
posterior
45°
horizontal canal
vertical canal
90°
20°
left
right

*Figure 7*

A theoretical polar plot for the left vertical canal. The circles lie along a horizontal axis set by the plane at the left canal. The silhouettes indicate the different orientations of the crab to the axis of rotation, which is parallel to the long axis of the page.

*Figure 8*

A theoretical polar plot for the right vertical canal. The circles lie along a horizontal axis set by the plane of the right canal.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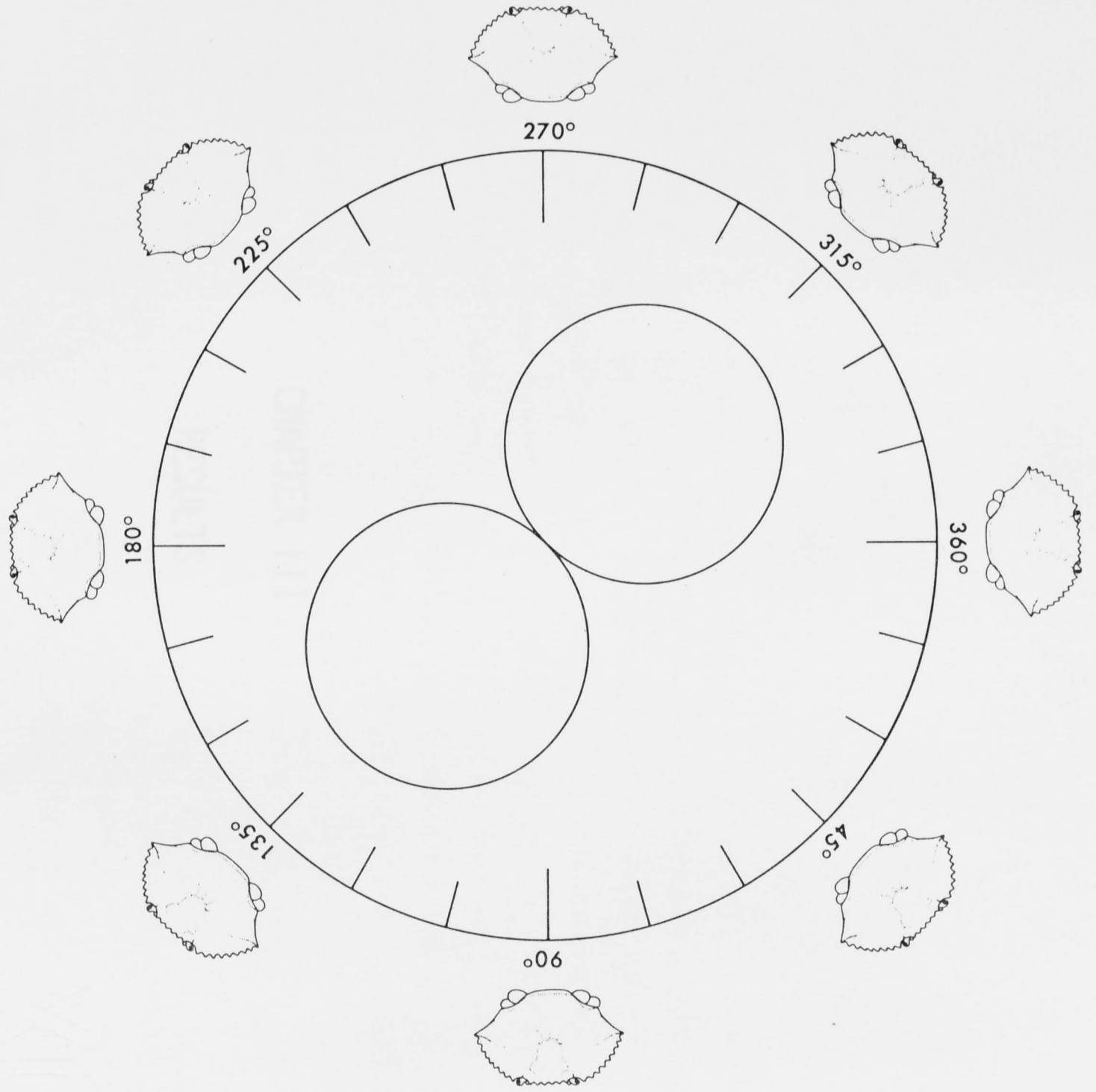
270°
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90°

# CHAPTER III

# RESULTS

Although many large mechanoreceptive and visual fibres were found during this study the only units considered are those showing constant or slowly adapting non-visual responses to rotation. The many rapidly-adapting units responding only during the first two or three cycles of pendulum oscillation have been omitted. Most units showed tactile responses and in some cases visually-mediated effects as well. These responses were investigated only far enough to demonstrate bi- or multimodality, proving that the fibres were not primary sensory afferents, and to reveal any gating effects.

Because of the variety of responses each unit has been described separately. The variables of particular interest have been tabulated for each unit and information important to the consideration of the directionality is presented graphically and is summarised at the end of the results section.

To facilitate comparison, units of similar properties have been arranged sequentially. Units I and II have tonic responses to maintained tilt. Units III to V respond to movement in a virtually non-directional manner. Unit VI responds to movement in both directions in all vertical planes, but shows a directionality which is very dependent on orientation. The remaining units all show strongly orientation-dependent directionality with only one direction excitatory. Within this grouping units are also ordered according to properties such as basic discharge, but it will be readily obvious that it is impossible to classify units in a completely consistent manner.

Unit I

| | |
|---|---|
| Recorded from | right tract |
| Basic discharge (horizontal position) | 5.5 $sp.sec^{-1}$, but variable |
| Static tilt | orientation-dependent tonic discharge varying with degree and direction of tilt. Strongest effects in 30° orientation. |
| Continuous oscillation | orientation-dependent phasic response with excitation in one direction, inhibition in the other. |
| Region of apparent phase change | 315-330° 135-150° |
| Phase in 45° orientation | 75° lead on peak hd/rsd position (range 54°-91°). |
| Tactile responses | excited by upper carapace stimulation |
| Visual responses | none evident |

Unit I is one of only two units shown to have a clear tonic discharge varying with the degree of maintained tilt. The other such unit, number II, was found in the same trace.

The low firing rate in the horizontal position varied spontaneously for no apparent reason, sometimes reaching twice the value given in the table above. The unit usually fired in doublets. Stimulation of the upper carapace with a paintbrush elicited a discharge of 21 $sp.sec^{-1}$ averaged over 3.5 seconds.

When the animal was held in a maintained displacement from horizontal the firing rate altered, being either inhibited or excited depending on the direction of the tilt. Figure 1 shows the response when the animal was tilted 15° from the horizontal and the orientation altered progressively from 0° to 360°. That is, the apparatus was deflected 15° right-side-down in the roll plane (0/360° orientation) and then the revolving plate rotated clockwise in 15° steps so that in the 90° orientation (Fig. 9) the animal is pitched head down, is rolled left-side-down at 180° and pitched head-up in the 270° position. The mean rate of dis-

charge in the first four seconds has been plotted. The response is most strongly excitatory in the 30° orientation, a combination of head-down and right-side-down. No values for orientations 345° and 360° (final) have been plotted as large spontaneous excitations exceeding 40 sp.sec$^{-1}$ occurred while held in the maintained position, and no steady values were obtained. The response was completely inhibited in orientations 120° to 315° inclusive.

The response to different degrees of maintained tilt in the pitch orientation is shown in Figure 10. There is an approximately linear relationship between degrees of tilt and rate of discharge, with the head-down displacement being excitatory. The frequency has been calculated over 4 seconds, during which time slight adaptation was apparent. A similar experiment in the roll plane showed excitation in the right-side-down positions and inhibition on right-side-up, but both effects were weak.

All static position units will show phasic responses to oscillations. Figure 11 shows the response of unit I to 20° ptp, 0.56 Hz oscillations in the 45° orientation. Broadly, an excitatory response occurs to right-side-down/head-down movement, and an inhibition occurs during rotation in the opposite direction. At this frequency and amplitude of oscillation the peak response led the peak right-side-down/head-down displacement by 75° (mean of five cycles, range 54°-91°). The positions of apparent phase change (Table above) indicate that the 45° orientation is the most directionally sensitive.

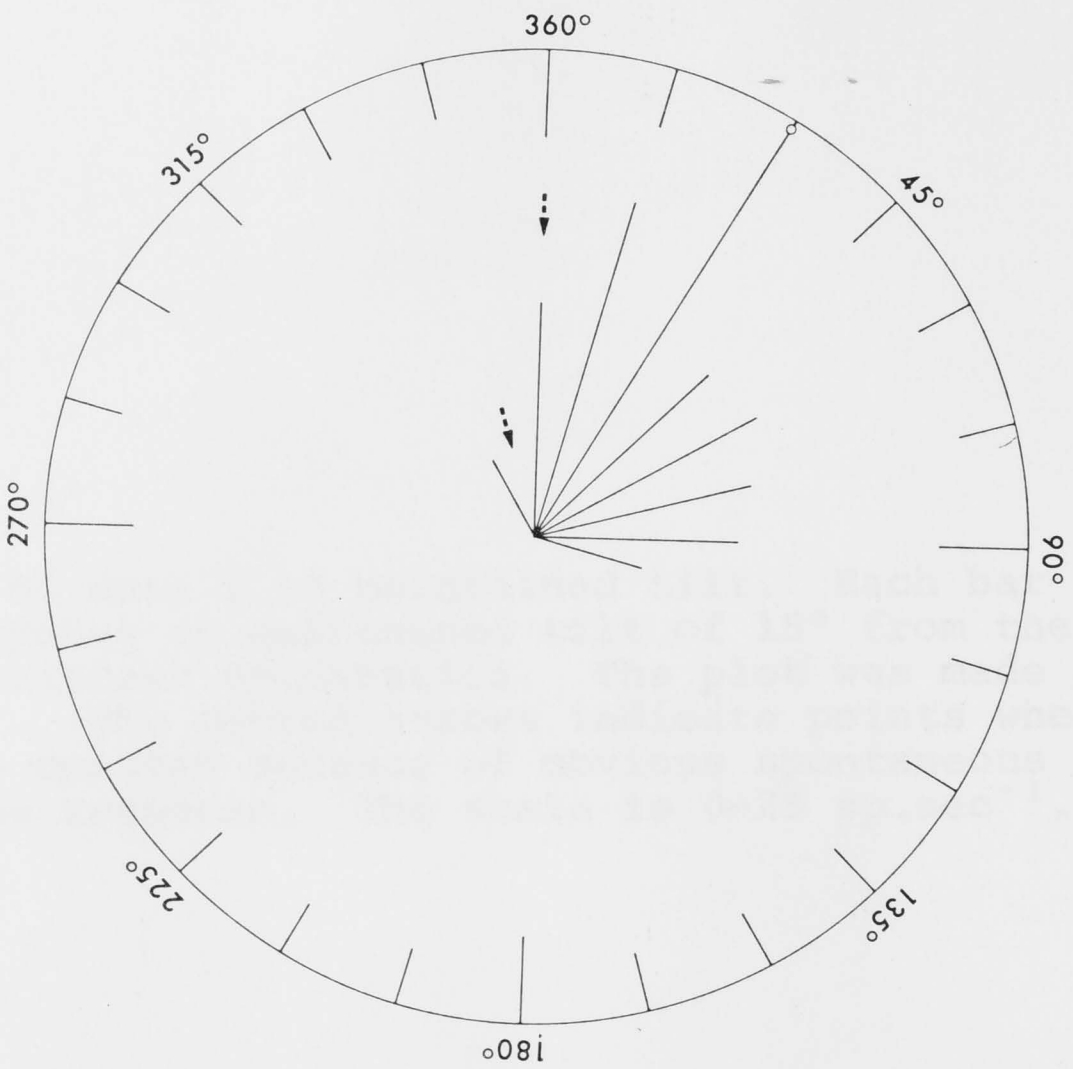
| Unit II | |
|---|---|
| Recorded from | right optic tract |
| Basic discharge rate (horizontal position) | 7-8 sp.sec$^{-1}$ |
| Static tilt | tonic output varied with orientation of animal |

*Figure 9*

The response of unit I to maintained tilt. Each bar represents the response to maintained tilt of 15° from the horizontal in a different orientation. The plot was made the sense 0° → 360°. The dotted arrows indicate points where readings have been omitted because of obvious spontaneous fluctuations in the response. The scale is 0-25 $sp.sec^{-1}$.

*Figure 10*

The response of unit I to varying degrees of maintained tilt in the pitch plane.


360°
45°
90°
135°
180°
225°
270°
315°

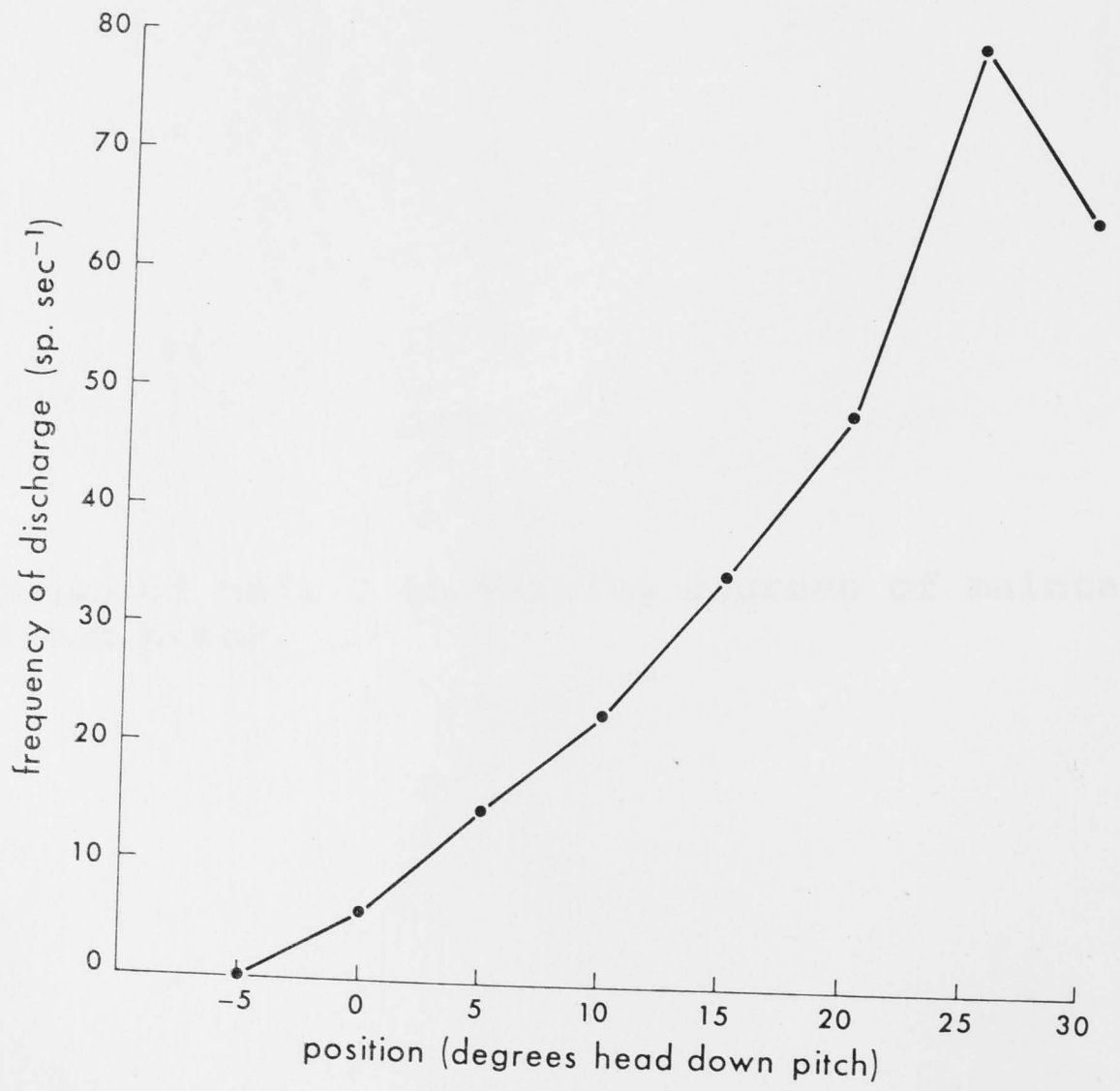
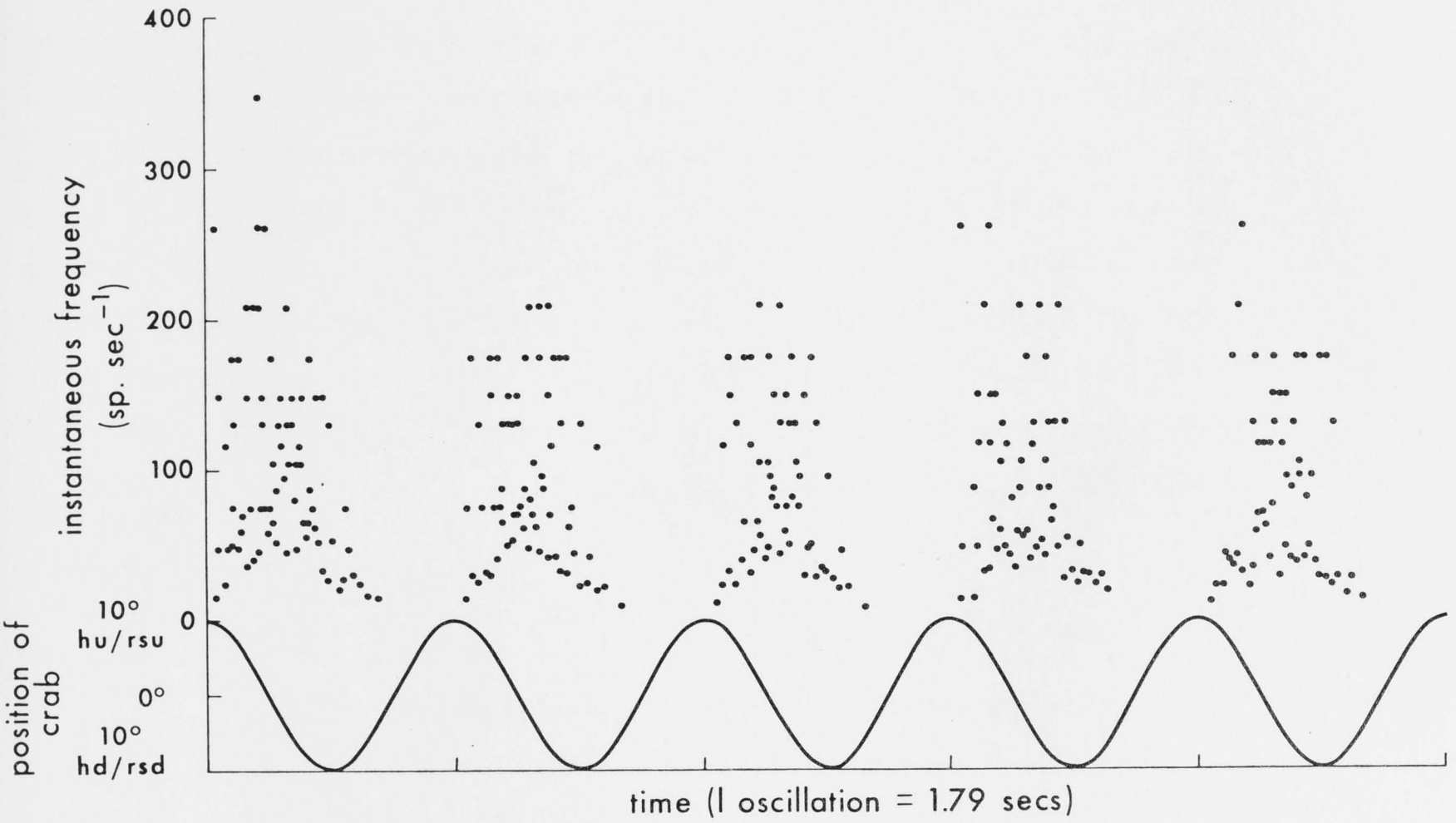

frequency of discharge (sp. sec⁻¹)
80
70
60
50
40
30
20
10
0
−5
0
5
10
15
20
25
30
position (degrees head down pitch)

*Figure 11*

The response of unit I to sinusoidal oscillation of 20° ptp at 0.56 Hz in the 45° orientation.


400
300
200
100
0
instantaneous frequency
(sp. sec$^{-1}$)
10°
hu/rsu
0
0°
10°
hd/rsd
position of crab
time (1 oscillation = 1.79 secs)

A small unit with a slow tonic discharge was recorded in the same preparation as unit I. A signal of sufficient amplitude to allow analysis was obtained only long enough for a polar plot to be determined (Fig. 12). The plot was made by deflecting the pendulum 15° rsd in the roll plane (orientation 0°/360°) and then turning the revolving plate through 360° in 15° intervals, recording the tonic discharge during a four second interval in each maintained position. The recording was made simultaneously with the plot for Unit I. The crab is right-side-down at 0°/360°, left-side-down at 180°, pitched head-down at 90° and head-up at 270°. The resting discharge was 7.0 $sp.sec^{-1}$ before and 8.3 $sp.sec^{-1}$ after the plot was made. The strongest response appears to be to left-side-down roll, a finding confirmed in other tests, where the maintained 25° lsd position gave a discharge rate of more than 25 $sp.sec^{-1}$, and 15° rsd roll gave a rate of less than 4 $sp.sec^{-1}$.

Unit III

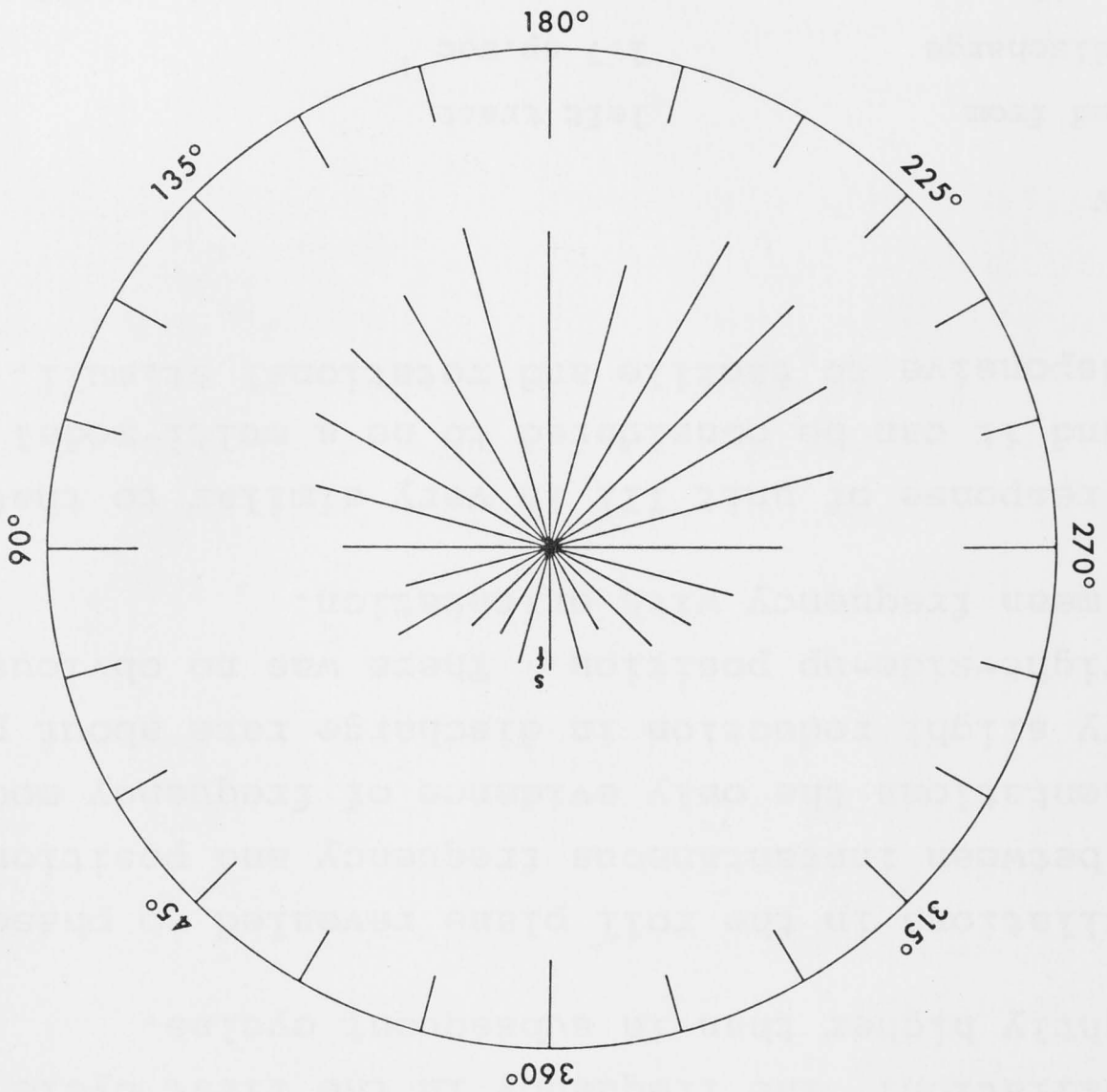
| | |
|---|---|
| Recorded from | right tract |
| Basic discharge rate | 3.2 $sp.sec^{-1}$ |
| Static tilt | no sustained effect |
| Continuous oscillation | all planes of rotation excitatory but non-directional, yaw strongly excitatory |
| Tactile input | phasic response to upper carapace stimulation |
| Visual input | experiments performed with eyes covered by weakly-translucent wax |

The stimulus to the carapace with a paintbrush elicited a phasic burst which reached frequency of 30 $sp.sec^{-1}$, but the rate dropped back to 3.2 $sp.sec^{-1}$ over 4 seconds immediately after the stimulus was discontinued.

There was no response to maintained tilt of 10° either side of horizontal in the roll plane. Movement to the tilt

*Figure 12*

The response of unit II to 15° maintained tilt in different orientations. The plot was made in the sense 0° → 360° and the starting and finishing values were almost identical. The scale is 0-12.5 $sp.sec^{-1}$.


180°
135°
225°
90°
270°
45°
315°
360°

position was excitatory, particularly in the counter-clockwise (rsd) sense, but the rate declined rapidly when the movement ceased.

Sinusoidal oscillations were excitatory with a maintained discharge of about 8 $sp.sec^{-1}$ regardless of orientation. Although the unit showed some adaptation over 70 cycles, this was probably attributable to decay in the pendulum oscillation. The frequency in the first cycle was only slightly higher than in subsequent cycles.

Oscillations in the roll plane revealed no phase relationship between instantaneous frequency and position. In other orientations the only evidence of frequency modulation was a very slight reduction in discharge rate about peak head-up/right-side-up position. There was no obvious alteration of mean frequency with orientation.

The response of unit III is very similar to that of unit IV and it can be considered to be a multi-modal interneuron responsive to tactile and rotational stimuli.

Unit IV

| | |
|---|---|
| Recorded from | left tract |
| Basic discharge | 1.7 $sp.sec^{-1}$ |
| Static tilt | no response to maintained tilt |
| Continuous oscillation | yaw strongly excitatory, rotations in vertical planes weakly excitatory. All non-directional. |
| Tactile response | phasic response to upper carapace stimulation. Habituates rapidly. |

Both rotation and tactile input excited this unit. In response to tactile stimulation of the upper carapace with a paintbrush the very low basic firing rate (1.7 $sp.sec^{-1}$) increased to 17 $sp.sec^{-1}$. Habituation was rapid, the fourth stroke within 5 seconds eliciting only two spikes.

Oscillations in the yaw plane were strongly excitatory but non-directional. In the vertical planes the response to oscillation was very uniform. The first cycle in each orientation gave a much stronger response than any other cycle, although the average frequency during the cycle was only 10 $sp.sec^{-1}$ approximately. By the sixth cycle the rate had adapted to about 4 $sp.sec^{-1}$, the effect being independent of orientation. Arbitrarily dividing the cycles in two at the point of maximum pendulum excursion indicated an apparent directional bias, with the first half of each cycle averaging about 30% more spikes, but the independence of this phenomenon from orientation suggests that it was not a genuinely directional effect. The total number of spikes in a run did not vary with the orientation in any systematic manner.

Pooled instantaneous frequency data in the 360° orientation did not reveal any obvious frequency modulation with position, the instantaneous frequency only once exceeding 20 $sp.sec^{-1}$. A histogram relating the number of spikes in successive 20° intervals of the oscillation cycle revealed a tendency to fire at the velocity peaks in both directions, and a complete inhibition about peak right-side-up.

The only conclusion which can be made is that the unit responds to both movement and tactile input.

Unit V

| | |
|---|---|
| Recorded from | right tract |
| Basic discharge | < 1.0 $sp.sec^{-1}$ |
| Static tilt | no effect |
| Continuous oscillation | strong non-directional excitation independent of orientation |
| Tactile response | excitatory response from entire carapace, and also antennule flagella and bases |
| Visual response | none observed |

Unit V was the largest of several similar units recorded together. All responded with very phasic bursts to tactile stimulation of any part of the carapace and several of the appendages. Although maintained tilt caused no change in the rate of discharge, oscillation was a strongly excitatory stimulus. The main frequency of discharge during the first 3 cycles in the 360° orientation was 52 $sp.sec^{-1}$. This was modulated only to a very slight degree with a tendency towards inhibition at positions of zero velocity and slightly stronger responses approximately at the velocity peaks in both directions of movement. In the pitch orientations (90° and 270°) the inhibitions at positions of zero velocity were more pronounced, particularly at head-up (Fig. 13). There was a slight suggestion that head-up rotation was more strongly excitatory, than head-down in the 270° orientation (Fig. 13). The mean frequency did not alter systematically with the orientation and the observed variations were small. The first cycle in all orientations gave an obviously stronger response than subsequent cycles, which progressively gave slightly diminishing responses. It was not clear whether this was an habituation or a result of a decaying oscillation.

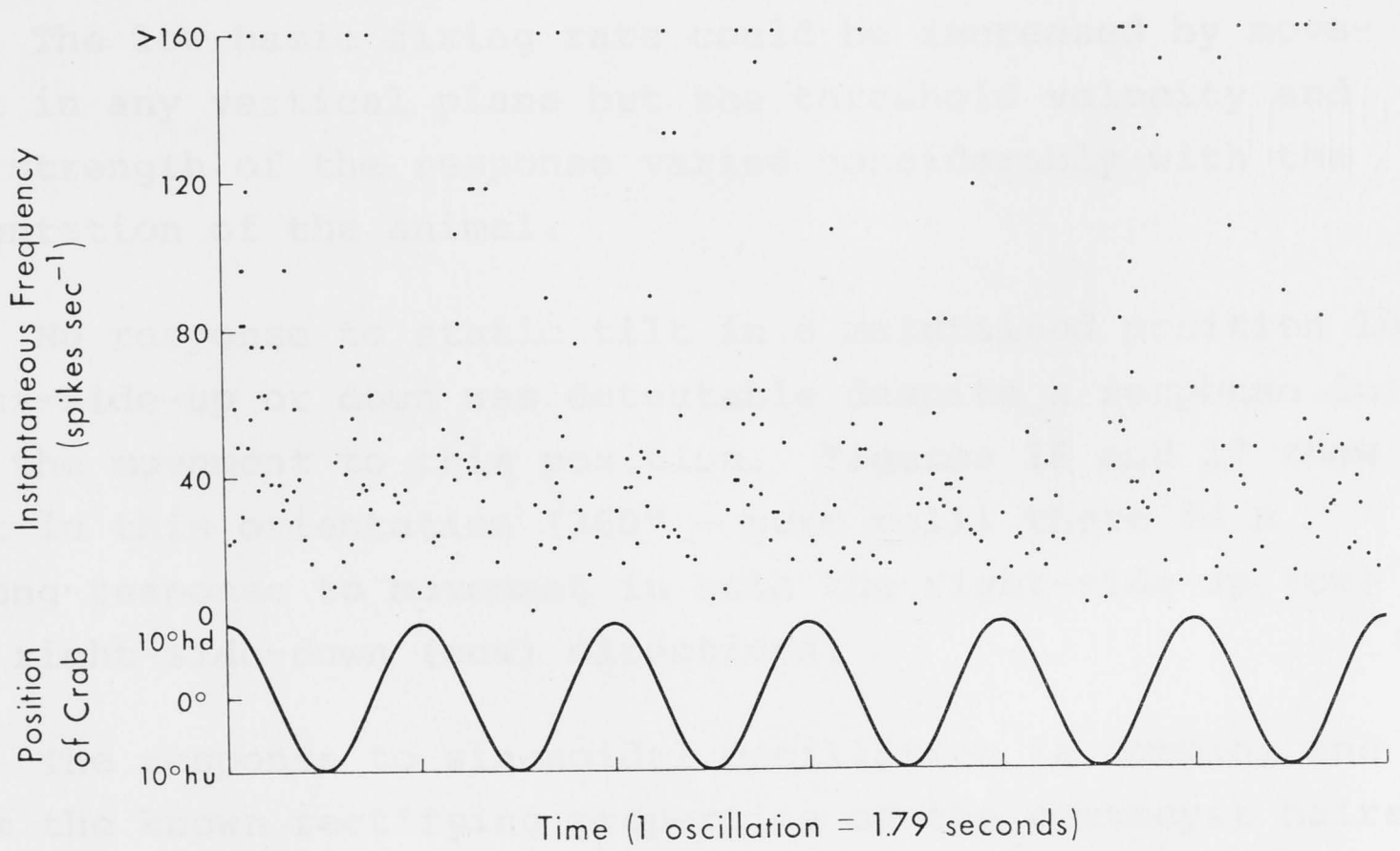
Unit V appears to be similar to units III and IV in most respects except race of discharge.

Unit VI

| | |
|---|---|
| Recorded from | right tract |
| Basic firing rate | < 0.5 $sp.sec^{-1}$ |
| Vestibular input | right (ipsilateral) statocyst. Probably also left. |
| Static tilt | no response |
| Continuous oscillation | general excitation with strong directional bias |
| Maximum directional bias | 75°-255° axis |
| Regions of apparent phase change (20° ptp osc.) | 60°-75°, 250°-255° |

*Figure 13*

The instantaneous frequency response of unit V to sinusoidal oscillation (20° ptp, 0.56 Hz) in the 270° orientation.


>160
120
80
40
0
Instantaneous Frequency (spikes sec$^{-1}$)
Position of Crab
10°hd
0°
10°hu
Time (1 oscillation = 1.79 seconds)

| | |
|---|---|
| Phase of major response in 75° and 255° orientations (20° ptp osc.) | 111° (range 101°-120) and 92.5° lead on head-down position |
| Tactile responses | phasic habituating response to carapace stimulation |
| Visual response | eyes covered over |

Although this obviously multimodal interneuron is rotation sensitive it does not appear to have canal input. Upon tactile carapace stimulation it showed rapidly adapting responses habituated upon repeated stimulation, but in one instance this stimulus elicited a sustained burst (> 35 sp.sec$^{-1}$) lasting more than 10 seconds. Similar bursts occasionally occurred spontaneously.

The low basic firing rate could be increased by movement in any vertical plane but the threshold velocity and the strength of the response varied considerably with the orientation of the animal.

No response to static tilt in a maintained position 10° right-side-up or down was detectable despite a response during the movement to this position. Figures 16 and 17 show that in this orientation (360° – pure roll) there is a strong response to movement in both the right-side-up (cw) and right-side-down (ccw) directions.

The response to sinusoidal oscillation is complex and from the known rectifying properties of the statocyst hairs in this animal (Janse and Sandeman, in preparation) it appears to require input from at least two hairs in rather different orientations. During oscillations at 20° ptp (peak-to-peak) the unit fires to both directions, with the relative strength of the response to the two directions being strongly dependent on the orientation of the animal. This is shown in Figure 14 where increasing length of the lines denotes a greater difference between the responses to the two directions. The greatest discrimination of direction occurs between 60° and 75° orientations and the 240°

and 255° orientations. These closely approach the pure pitch position and the major response occurs to head-down movement. The positions of apparent phase change (Fig. 14) confirm the above estimations of maximum directionality. The partial polar plot determined at a peak-to-peak amplitude of 10° indicates a similar directionality (Fig. 15) although the positions of apparent phase change have shifted.

The complexity of the response to movement is best illustrated in Figures 16 and 17. Figure 16 shows the mean response of the major half of each cycle (the half-cycle with the greater number of spikes). There is clearly no orientation-dependent variation inherent in this part of the response. Figure 17 shows the mean responses of the minor halves of the oscillations in each orientation and shows the greatest directionality approximately at right angles to that of Figure 14, which is obtained by subtracting the values in Figure 17 from those in Figure 16 (note that the scales are different).

In the positions showing greatest directional bias the minor cycle always has a response when the oscillation is 20° ptp (Fig. 17), but for 10° ptp oscillations this is not so. In many positions the minor half-cycle shows no response (Fig. 19b) and the recording appears to be from a typical rectifying rotation-sensitive unit. That the minor response is a clear excitatory response separate from the major response is shown in Figures 18 and 19a. In Figure 18 the weak excitatory response in the minor half is more or less continuous with the response of the major half in the first three cycles, but is clearly separate in the last three. Figure 19a shows the discrete responses clearly.

A plot of the instantaneous frequency of discharge during continuous oscillation in the 75° orientation is shown in Figure 19. It can be seen that the peak of the response to head-down movement clearly leads peak head-down position. In the 75° orientation the median spike method gives a 111°

mean phase lead at response on head-down position, or 21° phase lead on velocity in the head-down direction. In the 225° position the mean phase lead of 92.5° on head-down position, calculated for 20° ptp amplitude, is in close agreement with the value of 93° at 10° ptp amplitude. That is, the peak of the major discharge coincides very closely with the velocity peak in the head-down sense.

The first cycle in each orientation is always more strongly excitatory than the others. The first half-cycle always shows a strong response whether it is the major or the minor half, sometimes causing an apparent reversal in dominance during the first cycle in orientations where the directional bias is weak. The plot in Figure 14 should be symmetrical about a line drawn through the positions of apparent phase change. That it is not is partly a function of the effect of the first cycle. The half of the plot showing greater directionalities (330° → 150°) is the region where the major half of the cycle comes first, the effect of the first cycle increasing the apparent directional discrimination. Where the minor half-cycle occurs first the effect of the initial cycle tends to obscure the directional discrimination. Removing the left (contralateral) antennule base eliminated the minor response to roll (that is, the response to lsd rotation) and greatly weakened the major response. This suggests that the response to opposite directions of movement are mediated by different statocysts. In conflict with this conclusion is the observed diminution of the major cycle response, which suggests that the integrity of both statocysts is necessary to the maintenance of the sensitivity of the system, and without this any changes in directionality are obscured.

Neither the major nor the minor response is obtainable from canal units. The alignment of the minor cycle plot does not coincide with that of known canal interneurons (Fraser and Sandeman 1975). At first sight it appears possible to obtain the major cycle polar plot from combinations

of units from the two vertical canals (i.e. superimposing Figs. 7 and 8) but this requires excitatory input from both populations of thread hairs (responding to both upwards and downwards rotation) in both vertical canals. If this were the situation the minor cycle plot would be the same shape as the major cycle plot. Clearly this unit does not have canal input.

There was at least one other clear unit present in the trace. Its basic firing rate was variable but approximately the same as the unit described above. It responded to tactile stimuli in the same manner, but showed neither directional response to movement nor any constant phase between response and stimulus, although movement appeared to be weakly excitatory.

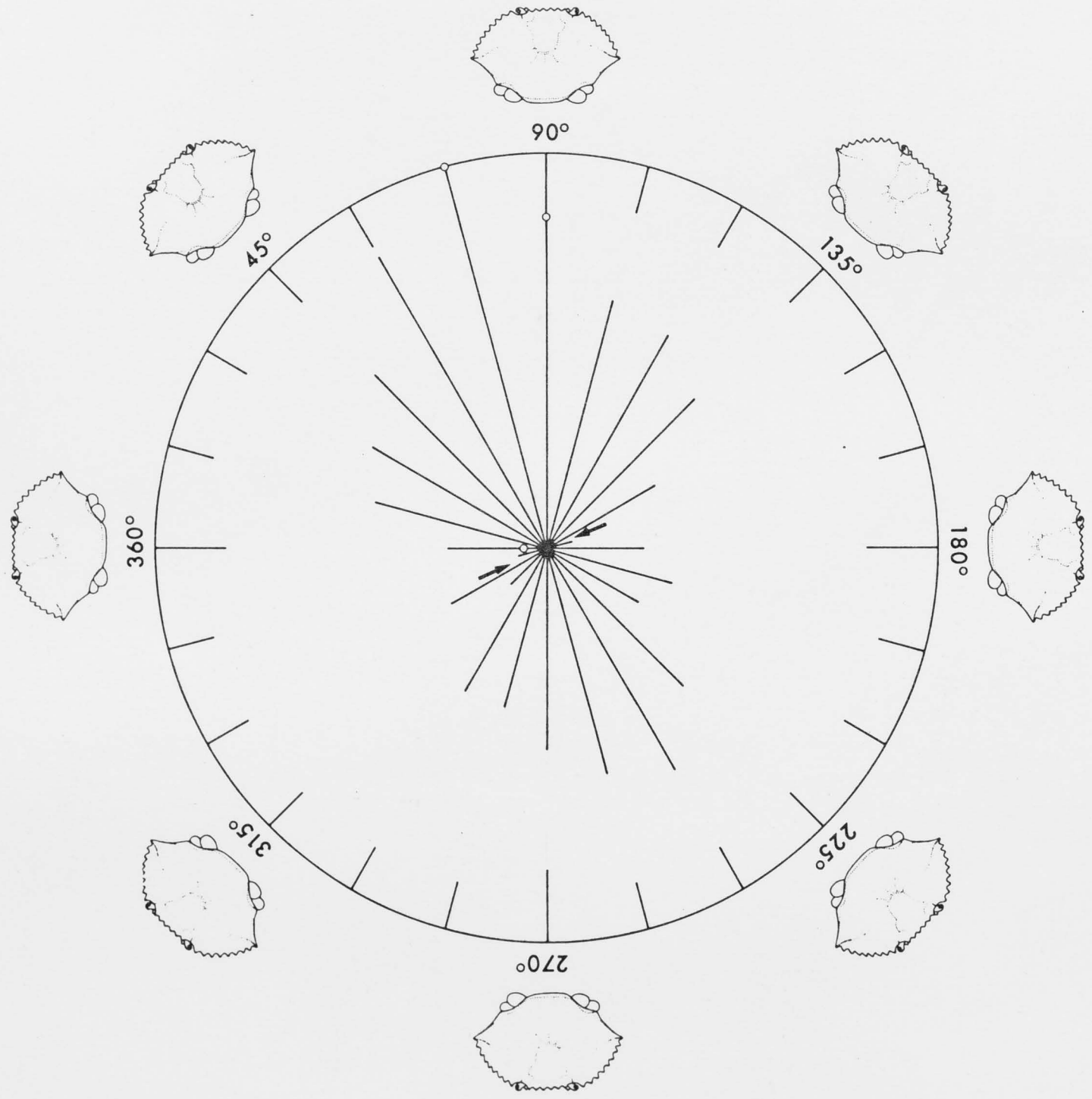
The directional information is clearly present but is the method of obtaining it valid? Counting absolute numbers of spikes over a given time interval reflects the frequency of the unit discharge and is a justifiable parameter to use in the absence of any indication of the importance of other properties of the spike train. In this and other studies (Fraser and Sandeman 1975) the number of spikes per cycle has been used in polar plots where there is either no response to movement in one direction because the unit is purely phasic with no resting discharge, or there is a modulation of a resting discharge, excitatory in one direction, inhibitory in the other. In both instances the statistic used is the difference between the number of spikes in two halves of the cycle. With the present unit there has been no change in procedure, but as the unit shows excitatory responses in both directions it is less certain that the animal is abstracting the directional information available.

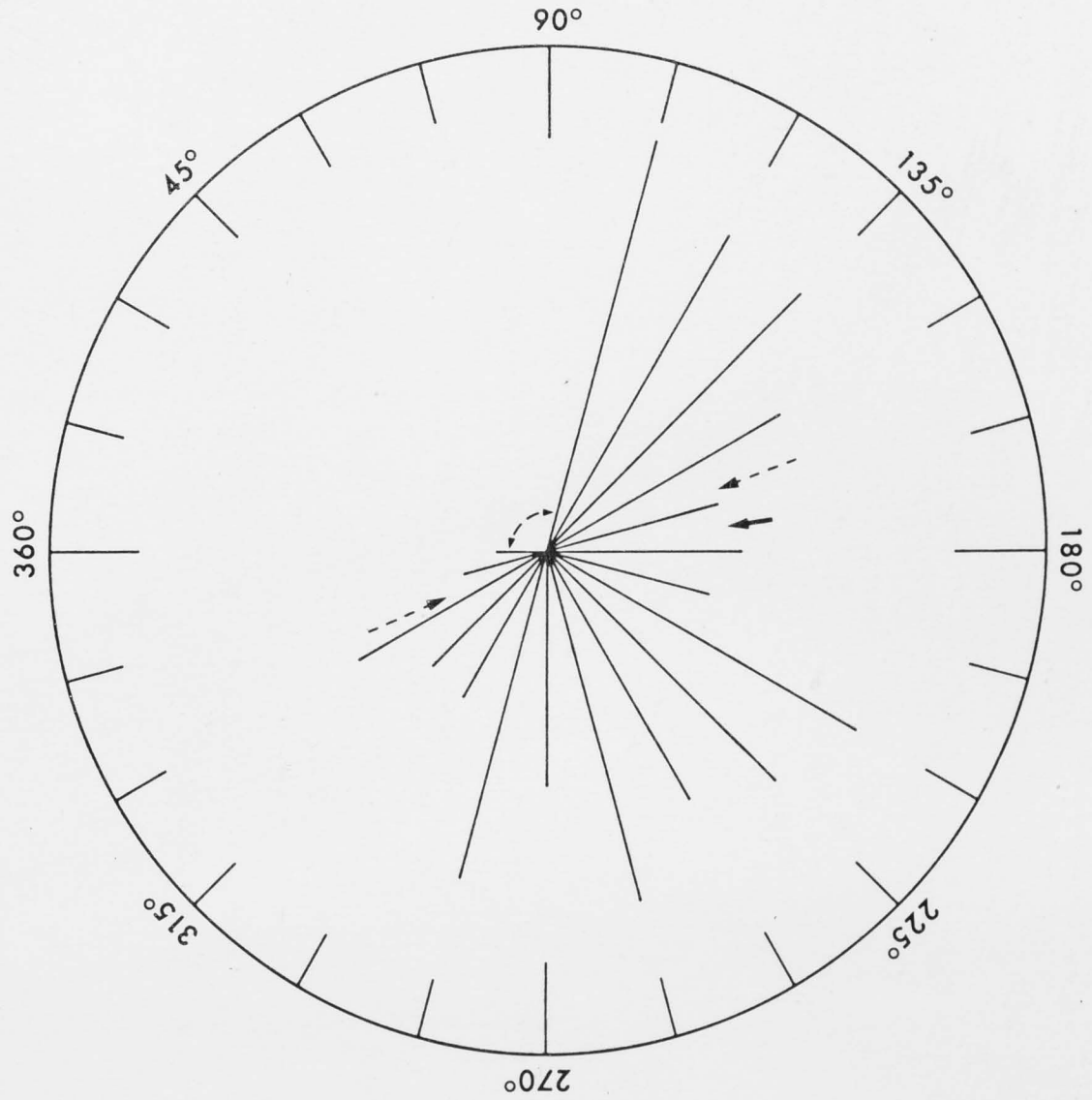
*Figure 14*

The directionality of the response of unit VI to sinusoidal oscillation (20° ptp, 0.56 Hz) in different orientations relative to the swing of the pendulum. The axis of oscillation is parallel to the long axis of the page. The silhouettes of crabs show the orientation of the crab when the corresponding value on the plot was determined. The length of the line equals the mean difference between the response (numbers of spikes) to movement in one direction and the movement in the opposite direction, determined during the first 6 cycles after the pendulum was released. The scale is 0-25 spikes from the centre to the perimeter of the plot. The solid arrows indicate the positions of apparent phase change. The open circle in the 360° orientation indicates the final determination (360° orientation) whereas the full length of the bar indicates the initial value (0° orientation). The values in orientation 75° and 90° are indicated by open circles. The plot was made in the sense 0° → 360°.

*Figure 15*

The directionality of the response of unit VI to sinusoidal oscillation (20° ptp, 0.56 Hz). The dotted line indicates untested orientations. The solid arrow indicates the position of apparent phase change. The dotted arrows indicate the positions of apparent phase change in the 20° ptp plot (Fig. 14). The length of the bar indicates the mean difference in the number of spikes in the two halves of cycles two to seven. Scale 0-25 spikes.




VI to sinu-
it orienta-
e axis of
age. The
crab when
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apparent
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value (0°
90° are
the sense

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indicates
arrows
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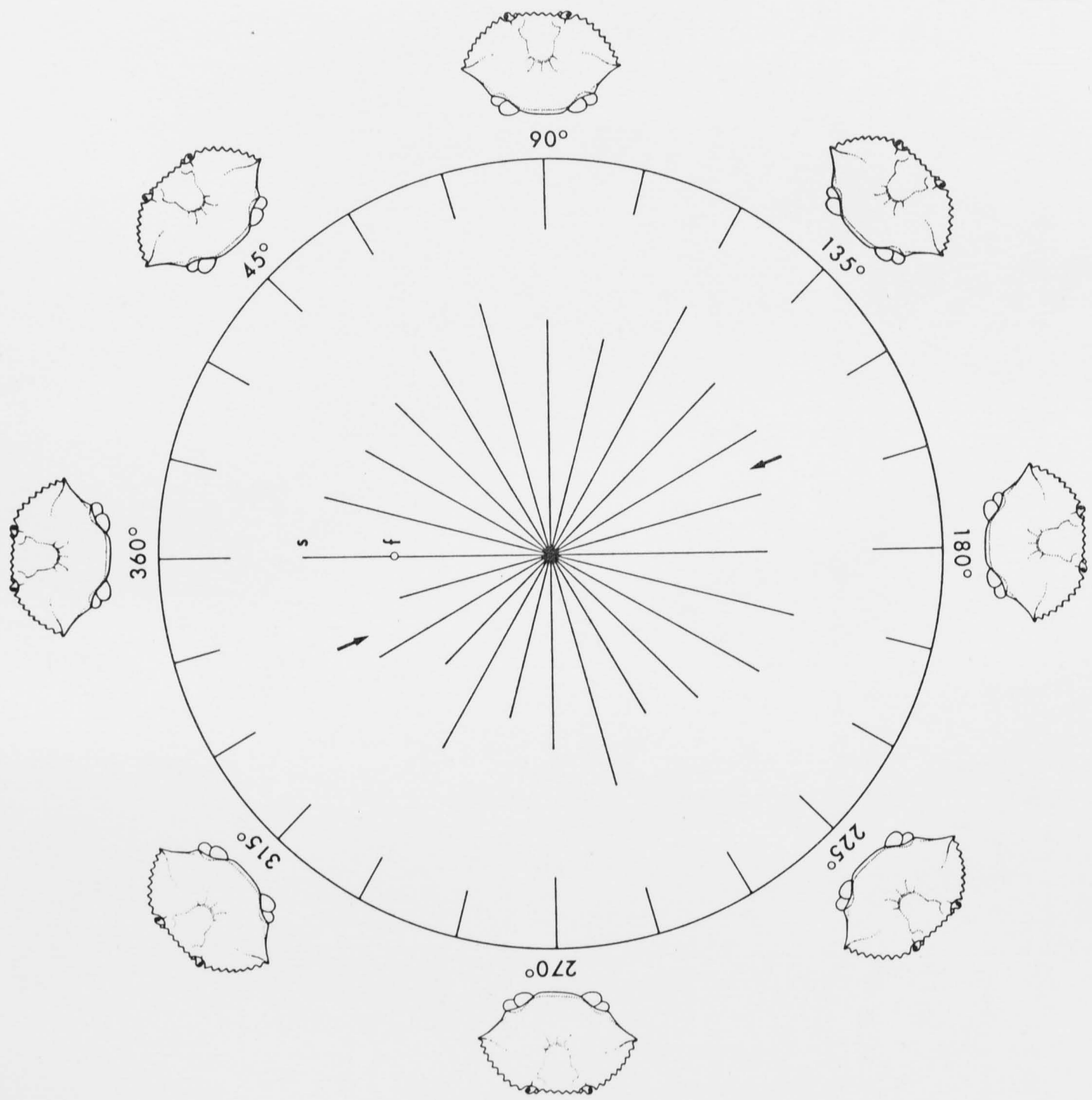
o halves

*Figure 16*

The mean number of spikes in the major half of the first six cycles in each orientation. Amplitude = 20° ptp. Scale 0-50 spikes. Solid arrows indicate the positions of apparent phase change. The open circle in the 360° orientation indicates the final value, the full length of the line the initial value.

*Figure 17*

The mean number of spikes in the minor half of the first six cycles in each orientation. Amplitude = 20° ptp. Scale 0-50 spikes. Solid arrows indicate the positions of apparent phase change. The open circle in the 360° orientation is the final value, the full length of the line the initial value.


90°
45°
135°
360°
180°
315°
225°
270°
s
f

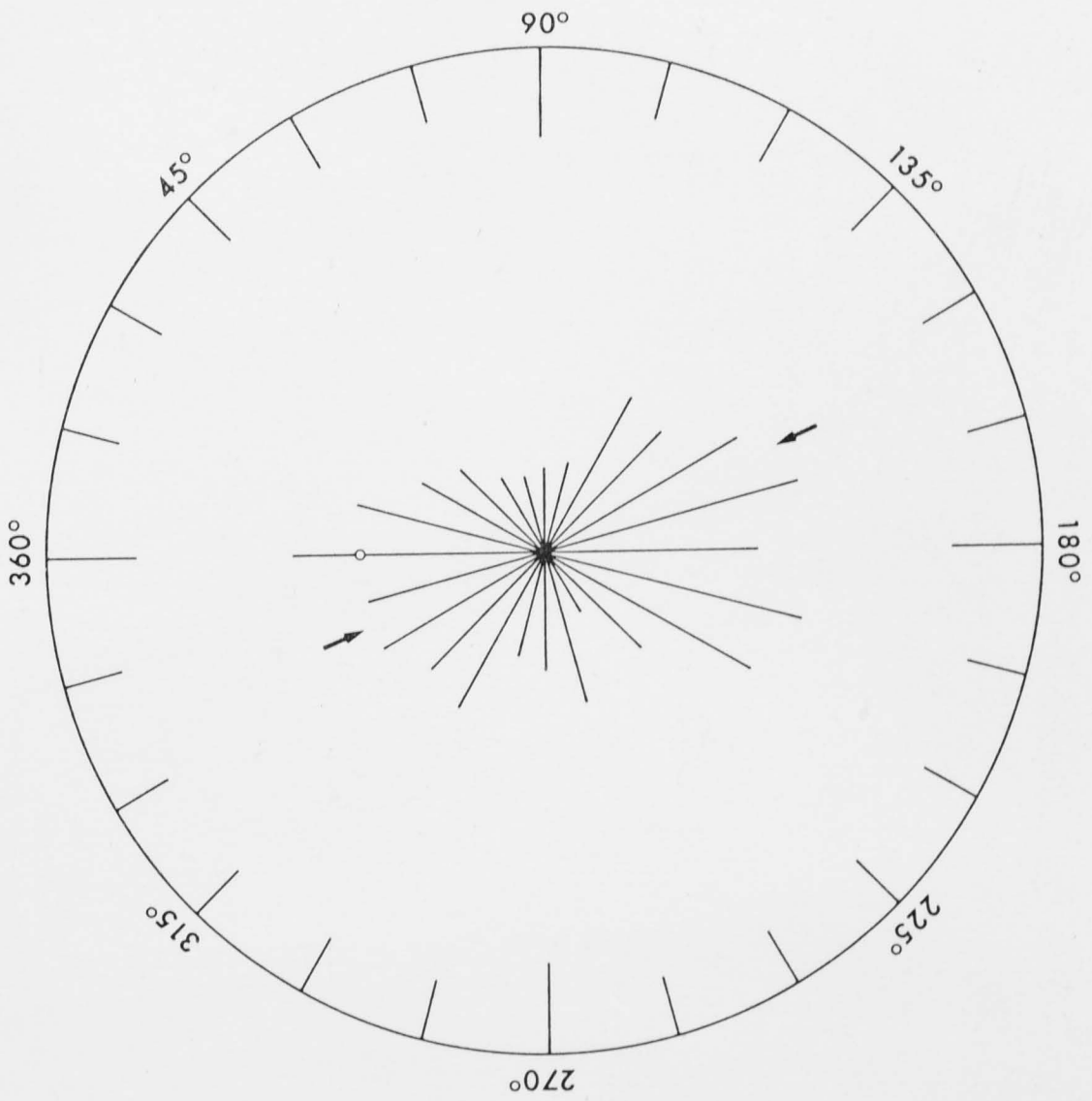

90°
45°
135°
360°
180°
315°
225°
270°

*Figure 18*

The instantaneous frequency response of unit VI to sinusoidal oscillation (20° ptp, 0.56 Hz) in the 75° orientation.

VI to
5° orient-

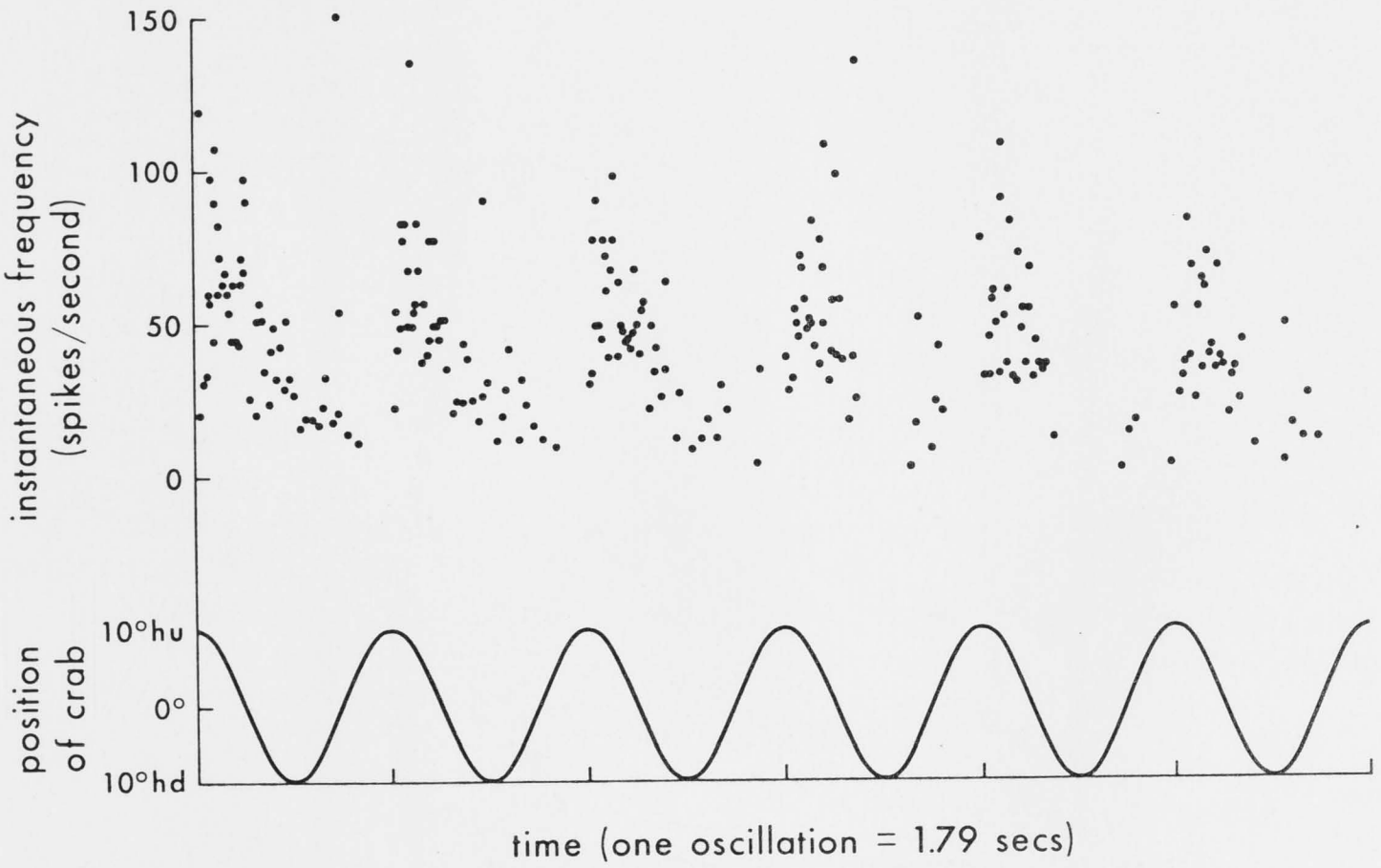


*Figure 19*

The response of unit VI when oscillated at 20° ptp (19a) and 10° ptp (19b) at 0.56 Hz in the 105° orientation. Trace up = head down. Released from the 10° head-up position.

a

b

Unit VII

| | |
|---|---|
| Recorded from | right optic tract |
| Basic discharge | no resting discharge |
| Static tilt | no response |
| Continuous oscillation | orientation-dependent, phasic directional response |
| Maximum directional bias | 180°-360° orientations |
| Region of apparent phase charge | 255°-270° |
| Phase in 180° orientation | 73° lead on rsd position (range 63°-82°) |
| Tactile response | occasional spike to vibration |
| Visual response | none apparent |

The strongly directional response of this unit is shown in Figure 20 where the mean number of spikes per cycle, averaged over the first six cycles, is the variable plotted. There was no response in either the 255° or 270° orientations, which are in the region of apparent phase change. In most orientations the unit adapted rapidly, although this was not invariable, as the following lists of the numbers of spikes per cycle show:

| Orientation | # of Spikes in Each Cycle | | | | | |
|---|---|---|---|---|---|---|
| 195° | 0 | 10 | 15 | 17 | 17 | 16 |
| 285° | 6 | 2 | 0 | 0 | 0 | 0 |
| 330° | 9 | 4 | 3 | 1 | 1 | 1 |
| 360° | 17 | 11 | 8 | 5 | 4 | 2 |

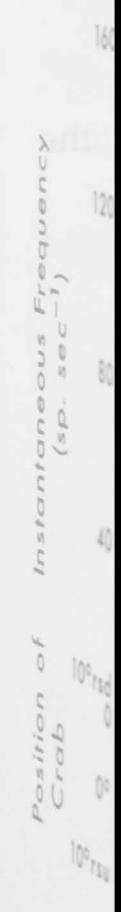
It appears that in those orientations where the first half-cycle was excitatory (orientations 255°-360° inclusive) the response to the initial cycle was the strongest and adaptation was rapid. Conversely, in the remaining orientations the response to the first cycle was weaker than the others and adaptation was not obvious, e.g. orientation 195° above

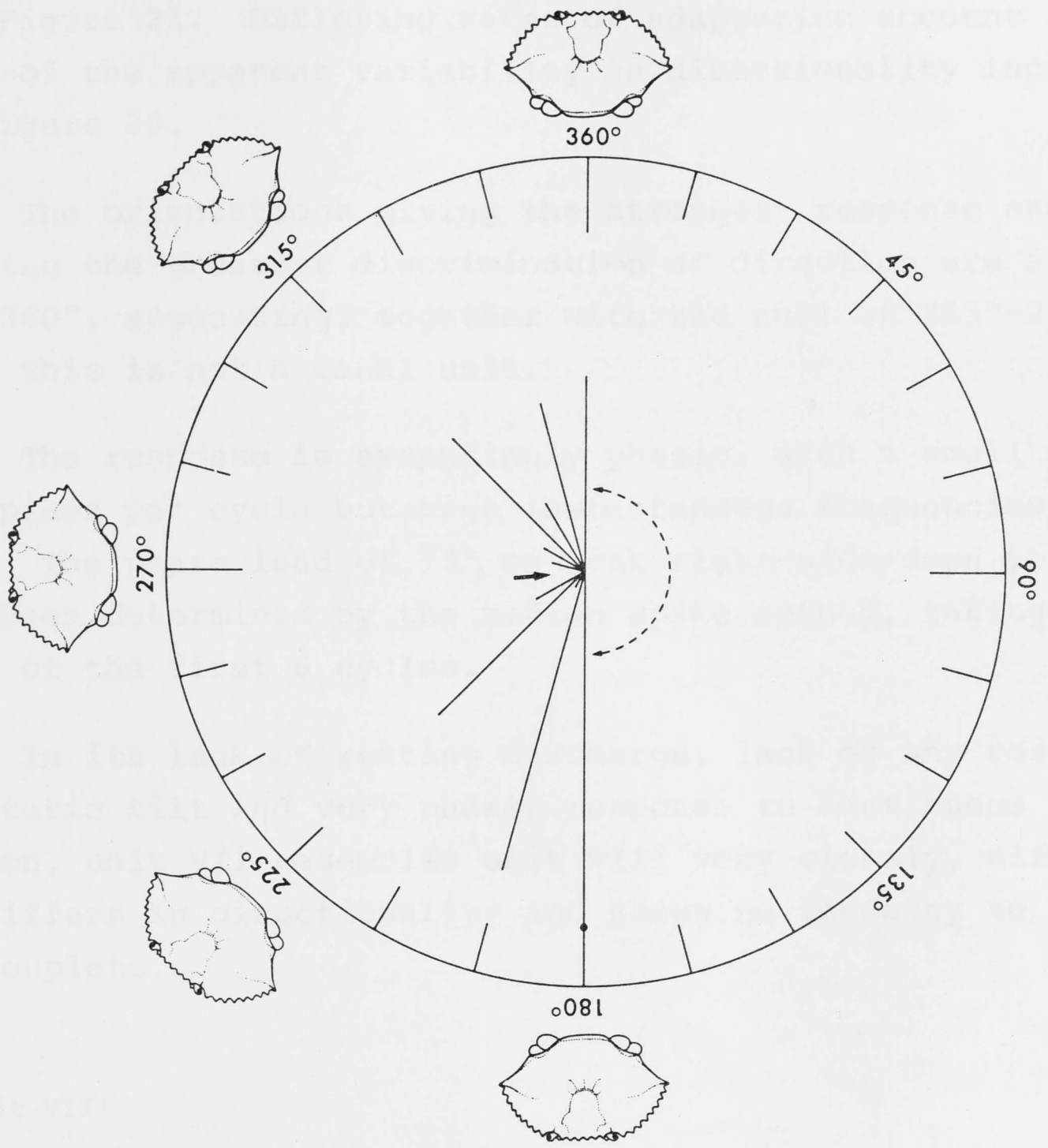
*Figure 20*

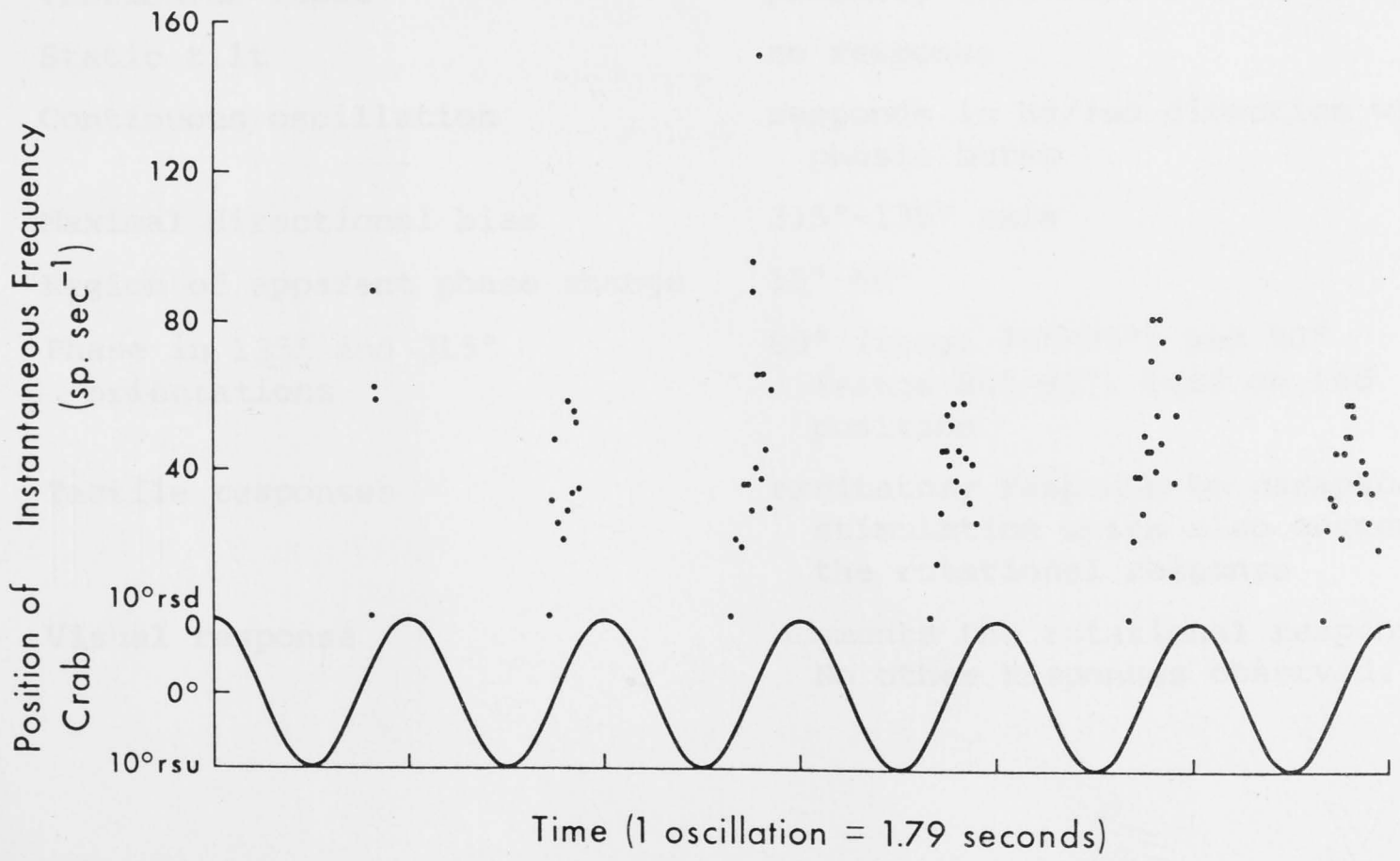
The directionality of the response of unit VII to sinusoidal oscillation (20° ptp, 0.56 Hz). The length of the bars indicates the mean number of spikes per cycle in the first 6 cycles in each orientation. Scale 0-16.7 $sp.cycle^{-1}$. The solid arrow indicates the position of apparent phase change. Responses were not determined in the region of the dotted line. The axis of rotation parallels the length of the page. The silhouettes indicate the different orientations of the crab, which was released from 10° right-side-up at 360°, and head-down at 270°. The solid circle in the 180° orientation indicates the magnitude of the response.

*Figure 21*

The instantaneous frequency response of unit VII to the first 6 oscillations after being released from 10° right-side-down in the 180° orientation. Pendulum frequency = 0.56 Hz.




360°
45°
90°
135°
180°
225°
270°
315°


160
120
80
40
10°rsd
0
0°
10°rsu
Position of Crab
Instantaneous Frequency (sp. sec$^{-1}$)
Time (1 oscillation = 1.79 seconds)

and Figure 21. Differing rates of adaptation account for much of the apparent variability in directionality indicated in Figure 20.

The orientations giving the strongest response and showing the greatest discrimination of direction are at 180° and 360°, suggesting, together with the null at 255°-270°, that this is not a canal unit.

The response is exceedingly phasic, with a small number of spikes per cycle but high instantaneous frequencies (Fig. 21). The phase lead of 73° on peak right-side-down position has been determined by the median spike method, taking the mean of the first 6 cycles.

In its lack of resting discharge, lack of any response to static tilt and very phasic response to continuous oscillation, unit VII resembles unit VIII very closely, although it differs in directionality and shows no tendency to fire in couplets.

Unit VIII

| | |
|---|---|
| Recorded from | right optic tract |
| Basic firing rate | 0 (no tonic discharge) |
| Vestibular input | probably left vertical canal |
| Static tilt | no response |
| Continuous oscillation | responds in hd/lsd direction with phasic burst |
| Maximal directional bias | 315°-135° axis |
| Region of apparent phase change | 15°-60° |
| Phase in 135° and 315° orientations | 89° (range 76°-95°) and 90° (range 86°-93°) lead on lsd position |
| Tactile responses | excitatory response to carapace stimulation which also augments the rotational response |
| Visual response | augments the rotational response. No other responses observed. |

Tactile stimulation of the carapace elicited a burst averaging 23 $sp.sec^{-1}$ over 3 seconds during stimulation, but ceasing soon after the stimulus was stopped. Apart from rotational movement, no other independently excitatory stimulus was detected.

Rotational movement elicited phasic bursts of spikes in a pattern suggesting that downwards rotation of the left vertical canal was the excitatory stimulus. The polar response in Figure 22 supports this suggestion. The region of apparent phase change listed in the table is broad because there are zero values in the 30° and 45° orientations. Another plot of the same unit not reproduced here showed a zero value only in the 30° orientation. The maximum responses, expressed as spikes per cycle, occurred in the 315° and 135° positions (Fig. 22) in good agreement with another determination giving values of 330° and 135°.

Figure 23 is a plot of the instantaneous frequency during 20° ptp oscillation in the 315° orientation. The apparent division of the response (Fig. 22) into either high (above 125 $sp.sec^{-1}$) or low frequencies (below 90 $sp.sec^{-1}$) is largely a result of the tendency of the unit to fire in pairs. The phase relationships in the 315° and 135° positions were 90° and 89° leads on hd/lsd position respectively. The mediar spike technique was used. The negligible difference between the two values is not what we expect to find from thread hair units (Fraser and Sandeman 1975, Janse and Sandeman, in preparation). The second determination gave leads of 93° and 68° in these same positions. Oscillation in the 135° orientation begins from a 10° hd position and the 315° orientation starts from 10° hd/lsd.

The variations in the discharge of the unit suggested above also contribute to variations in the precise form of the polar plot. In general the unit showed marked signs of adaptation by the fifth oscillation in any orientation, but the number of spikes per cycle varied considerably, both

increasing and decreasing, sometimes showing no adaptation at all after 8 cycles. Two different determinations of the response in the 75° orientation are given here (figures represent numbers at spikes per cycle).

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1st | 7 | 9 | 3 | 16 | 6 | 8 | 3 | 0 | 0 |
| 2nd | 39 | 30 | 24 | 27 | 22 | 22 | 23 | 19 | 11 |

The two sets together are the most extreme variations observed in one orientation. They were recorded about 15 minutes apart during which time similar tests were made in other orientations. Neither of these sets of figures is used in Figure 22.

While the variations discussed above were apparently spontaneous the addition of either visual or tactile (carapace) stimulation during the oscillation greatly enhanced the strength of the response. Listed below are the numbers of spikes per cycle for two runs made in the 315° position. In the first, tactile carapace stimulation is added after the fifth cycle (double lines). The second is made with a light shining on the eye for the first 5 cycles, after which it is turned off.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tactile | 32 | 39 | 37 | 38 | 35 | // | 76 | 57 | 51 | 36 | |
| | | | | | tactile input applied | | | | | | |
| Visual | 40 | 43 | 46 | 38 | 29 | // | 30 | 18 | 11 | 9 | 9 | 7 |
| | | | | | light off | | | | | | |

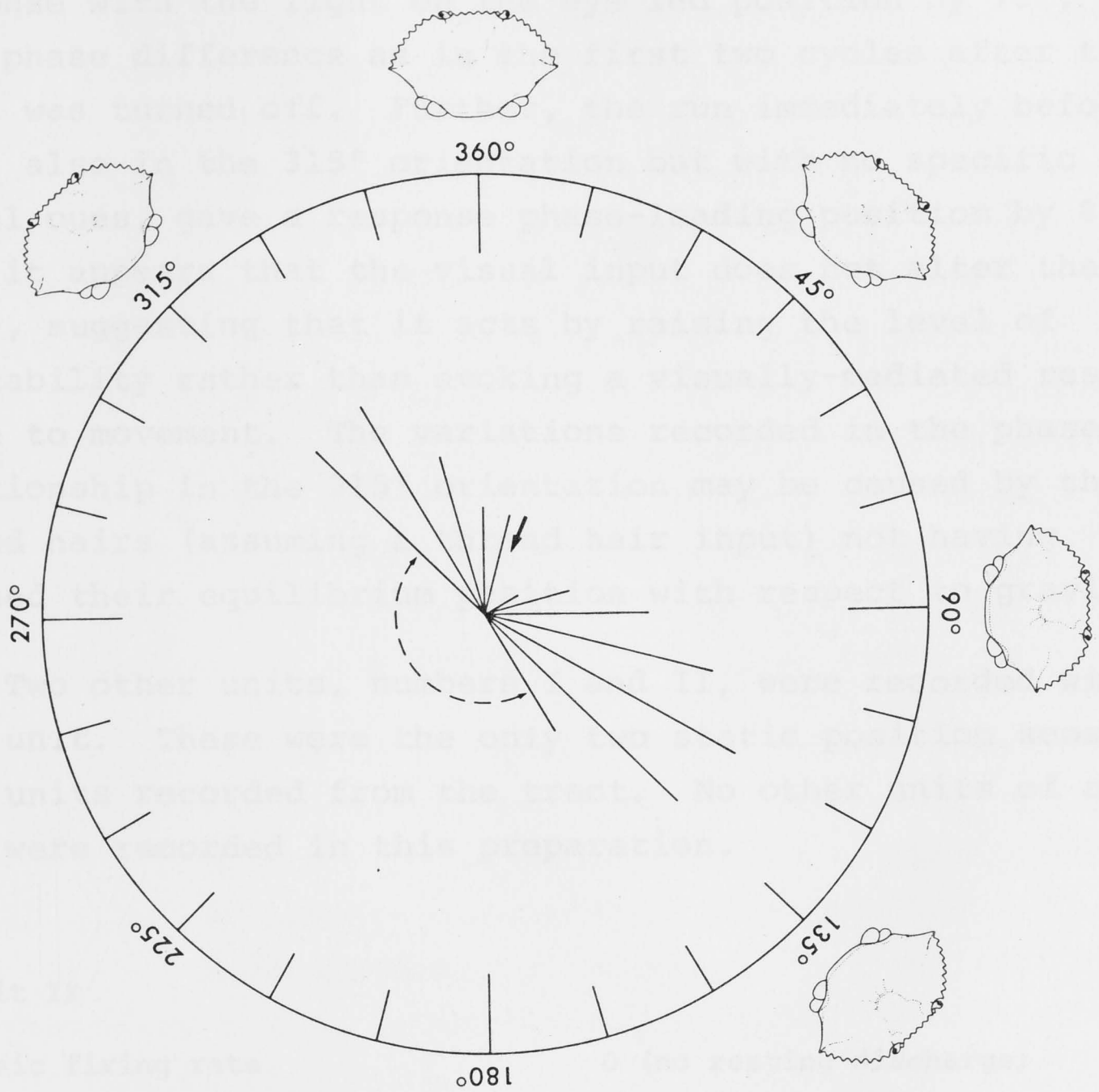
During the tactile input the response was continued over a large part of the first two cycles but still showed inhibition during rotation in the non-preferred direction. With the visual input there seems to be adaptation from a higher level of response, with light-off causing the response to diminish even further. It is not known whether the light acted simply to increase the excitability of the unit or added an obvious visual cue by which rotational movement could be measured. The unit did not respond to any purely visual input, but this was not exhaustively tested. The

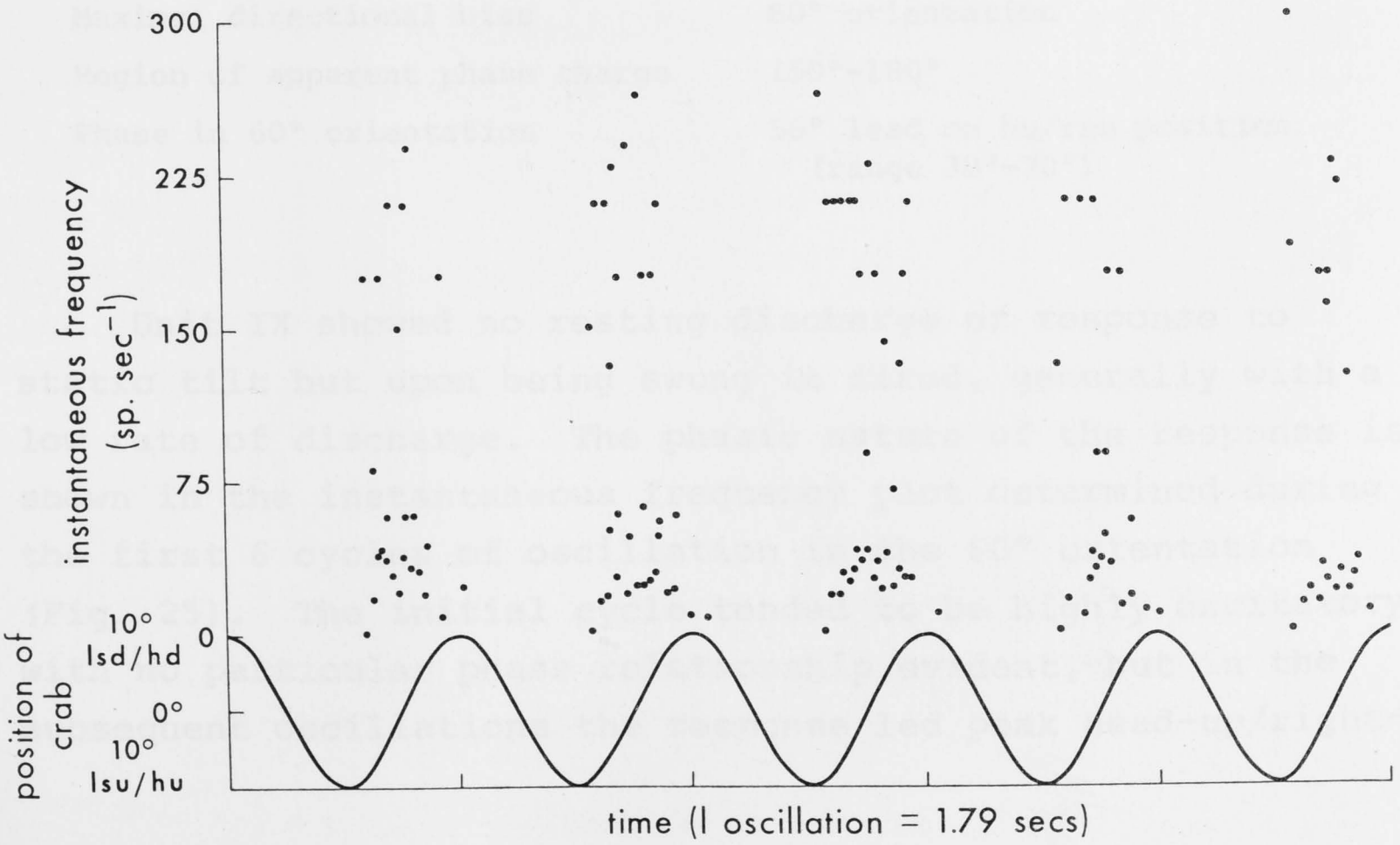
*Figure 22*

The directionality of the response of unit VIII to sinusoidal oscillation (20° ptp, 0.56 Hz) in different orientations, shown by the silhouettes around the graph. The axis of rotation was parallel to the long axis of the page. The solid arrow indicates the position of apparent phase change. The dotted line indicates orientations for which no values have been determined. The statistic plotted is "number of spikes per cycle" averaged over 5 cycles and the scale is 0-50 spikes along the radial axis.

*Figure 23*

The instantaneous frequency response of unit VIII to the first 5 cycles of sinusoidal oscillation (20° ptp, 0.56 Hz) in the 315° orientation.


360°
45°
90°
135°
180°
225°
270°
315°


300
225
150
75
0
instantaneous frequency
(sp. sec⁻¹)
10°
lsd/hd
0°
10°
lsu/hu
position of
crab
time (1 oscillation = 1.79 secs)

response with the light on the eye led position by 79°, the same phase difference as in the first two cycles after the light was turned off. Further, the run immediately before this, also in the 315° orientation but with no specific visual cues, gave a response phase-leading position by 82°. Thus it appears that the visual input does not alter the phase, suggesting that it acts by raising the level of excitability rather than evoking a visually-mediated response to movement. The variations recorded in the phase relationship in the 315° orientation may be caused by the thread hairs (assuming a thread hair input) not having reached their equilibrium position with respect to gravity.

Two other units, numbers I and II, were recorded with this unit. These were the only two static position sensitive units recorded from the tract. No other units of any sort were recorded in this preparation.

Unit IX

| | |
|---|---|
| Basic firing rate | 0 (no resting discharge) |
| Vestibular input | uncertain |
| Static tilt | no response |
| Continuous oscillation | general excitation with strong directional bias |
| Maximal directional bias | 60° orientation |
| Region of apparent phase charge | 150°-180° |
| Phase in 60° orientation | 56° lead on hu/rsu position (range 38°-70°) |

Unit IX showed no resting discharge or response to static tilt but upon being swung it fired, generally with a low rate of discharge. The phasic nature of the response is shown in the instantaneous frequency plot determined during the first 6 cycles of oscillation in the 60° orientation (Fig. 25). The initial cycle tended to be highly excitatory with no particular phase relationship evident, but in the subsequent oscillations the response led peak head-up/right-

side-up position by 56° (median spike method). Even when the unit fired only once per cycle the spike was usually in the region of 50°-60° lead on peak position. In the region of the apparent phase change the unit sometimes fired to movement in both directions for several cycles, but only to one direction during subsequent oscillations. This caused difficulties in determining the position of apparent phase change, which may actually be closer to the 150° orientation than where it is drawn in Figure 24. The statistic plotted in Figure 24 is the mean difference between the numbers of spikes in the two different directions of movement in each cycle, averaged over cycles two to seven to avoid distortions introduced by the effect of the first cycle.

While the phase relationship of the response to the discharge was approximately constant the number of spikes per cycle varied greatly, sometimes showing rapid adaptation, occasionally increasing on subsequent oscillations and sometimes showing markedly different strengths of response to two apparently similar stimuli, e.g. the 0/360° and 180° orientations in Figure 24.

The sensory input to unit IX is unclear, but the responses observed resembled those of unit VIII in that there was no resting discharge, the amplitude of the recorded potential was large (extracellular recording) and the maximal response occurred close to the optimal orientation for stimulation of a vertical canal, in this case the right. The several units found in this same trace behaved in a qualitatively similar manner to unit IX, but were too similar in size to permit independent analysis.

Unit X

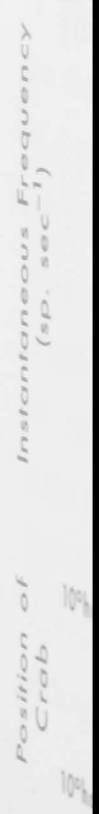
| | |
|---|---|
| Recorded from | left tract |
| Basic discharge | variable, mean < 1.0 $sp.sec^{-1}$ |
| Static tilt | no apparent response |

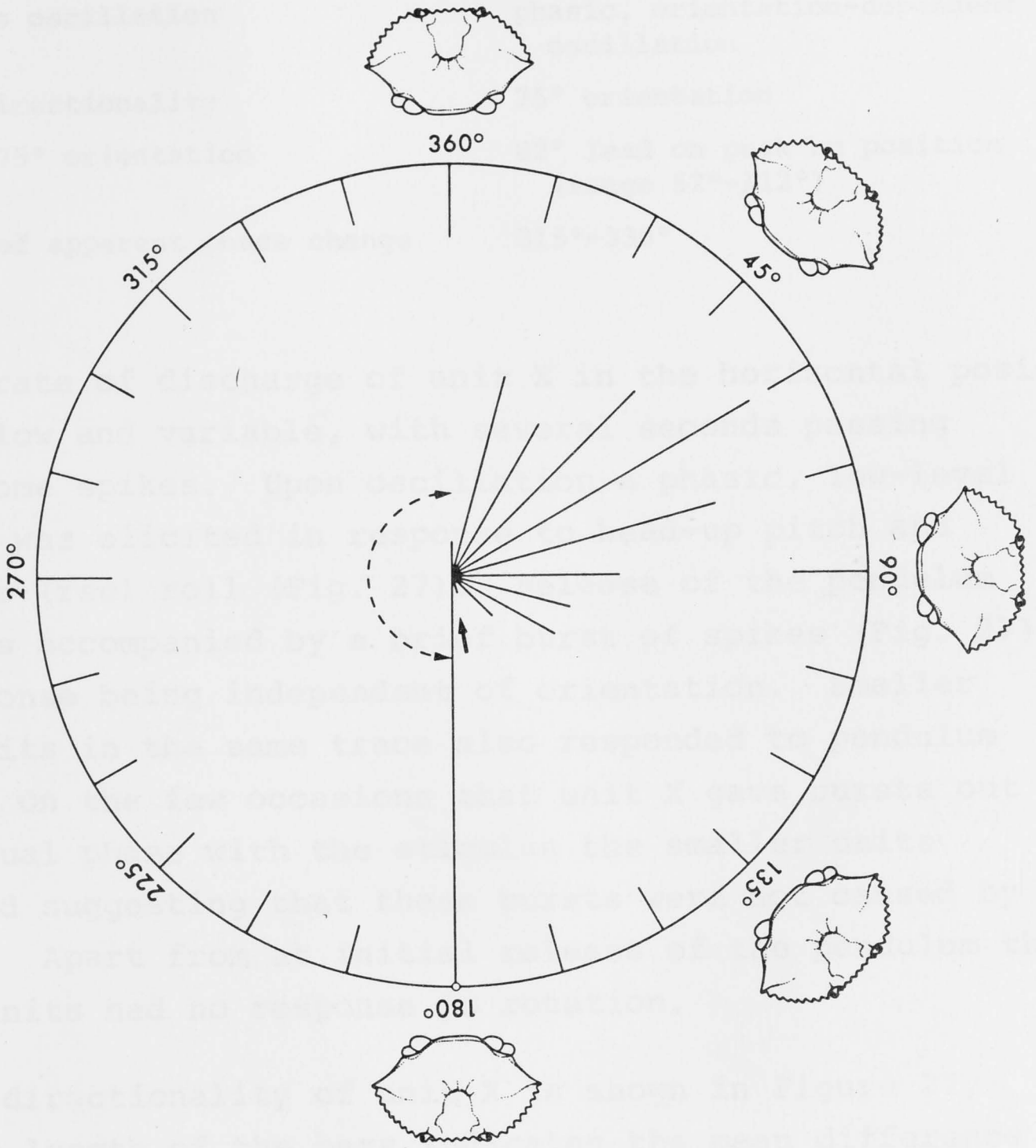
*Figure 24*

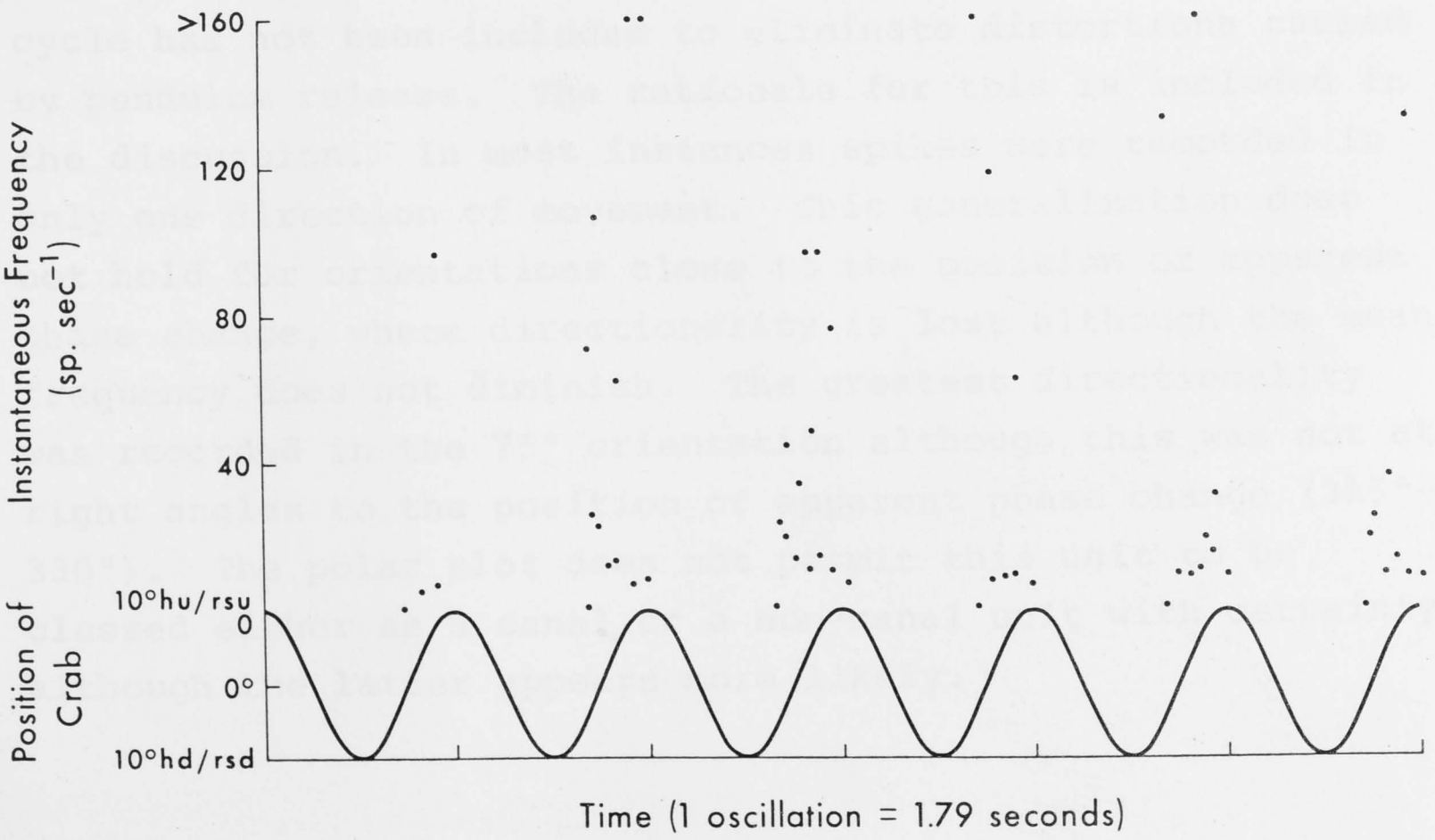
The directionality of the response of unit IX to 20° ptp sinusoidal oscillation in different orientations as shown by the silhouettes around the graph. The dotted arrow indicates the orientations not tested. The solid arrow shows the position of apparent phase change. The scale is 0-12.5 spikes. The value in orientation 180° actually reaches 15.0 spikes.

*Figure 25*

The instantaneous frequency response of unit IX to the first 6 cycles of sinusoidal oscillation (20° ptp, 0.56 Hz) in the 60° orientation.




360°
45°
90°
135°
180°
225°
270°
315°


>160
120
80
40
0
0°
Instantaneous Frequency
(sp. sec$^{-1}$)
Position of Crab
10°hu/rsu
10°hd/rsd
Time (1 oscillation = 1.79 seconds)

| | |
|---|---|
| Continuous oscillation | phasic, orientation-dependent oscillation |
| Maximum directionality | 75° orientation |
| Phase in 75° orientation | 82° lead on peak hu position (range 52°-112°) |
| Position of apparent phase change | 315°-330° |

The rate of discharge of unit X in the horizontal position was low and variable, with several seconds passing between some spikes. Upon oscillation a phasic, low-level discharge was elicited in response to head-up pitch and clock-wise (rsu) roll (Fig. 27). Release of the pendulum was always accompanied by a brief burst of spikes (Fig. 27), this response being independent of orientation. Smaller phasic units in the same trace also responded to pendulum release. On the few occasions that unit X gave bursts out of the usual phase with the stimulus the smaller units also fired suggesting that these bursts were not caused by rotation. Apart from at initial release of the pendulum the smaller units had no response to rotation.

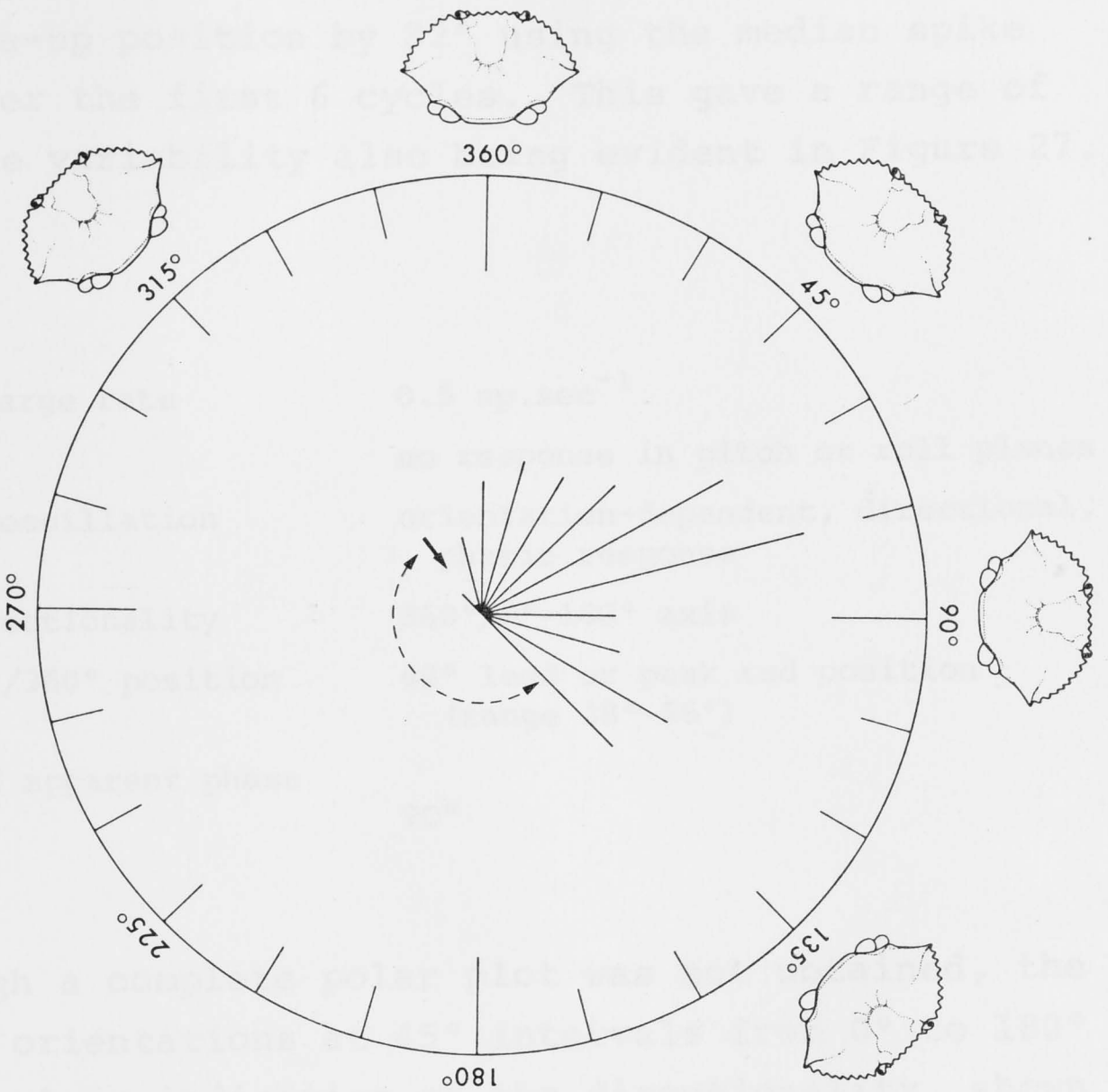
The directionality of unit X is shown in Figure 27, where the length of the bars indicates the mean difference in the number of spikes in the two directions of movement during cycles 2 to 6 after pendulum release. The initial cycle has not been included to eliminate distortions caused by pendulum release. The rationale for this is included in the discussion. In most instances spikes were recorded in only one direction of movement. This generalization does not hold for orientations close to the position of apparent phase change, where directionality is lost although the mean frequency does not diminish. The greatest directionality was recorded in the 75° orientation although this was not at right angles to the position of apparent phase change (315°-330°). The polar plot does not permit this unit to be classed either as a canal or a non-canal unit with certainty, although the latter appears more likely.

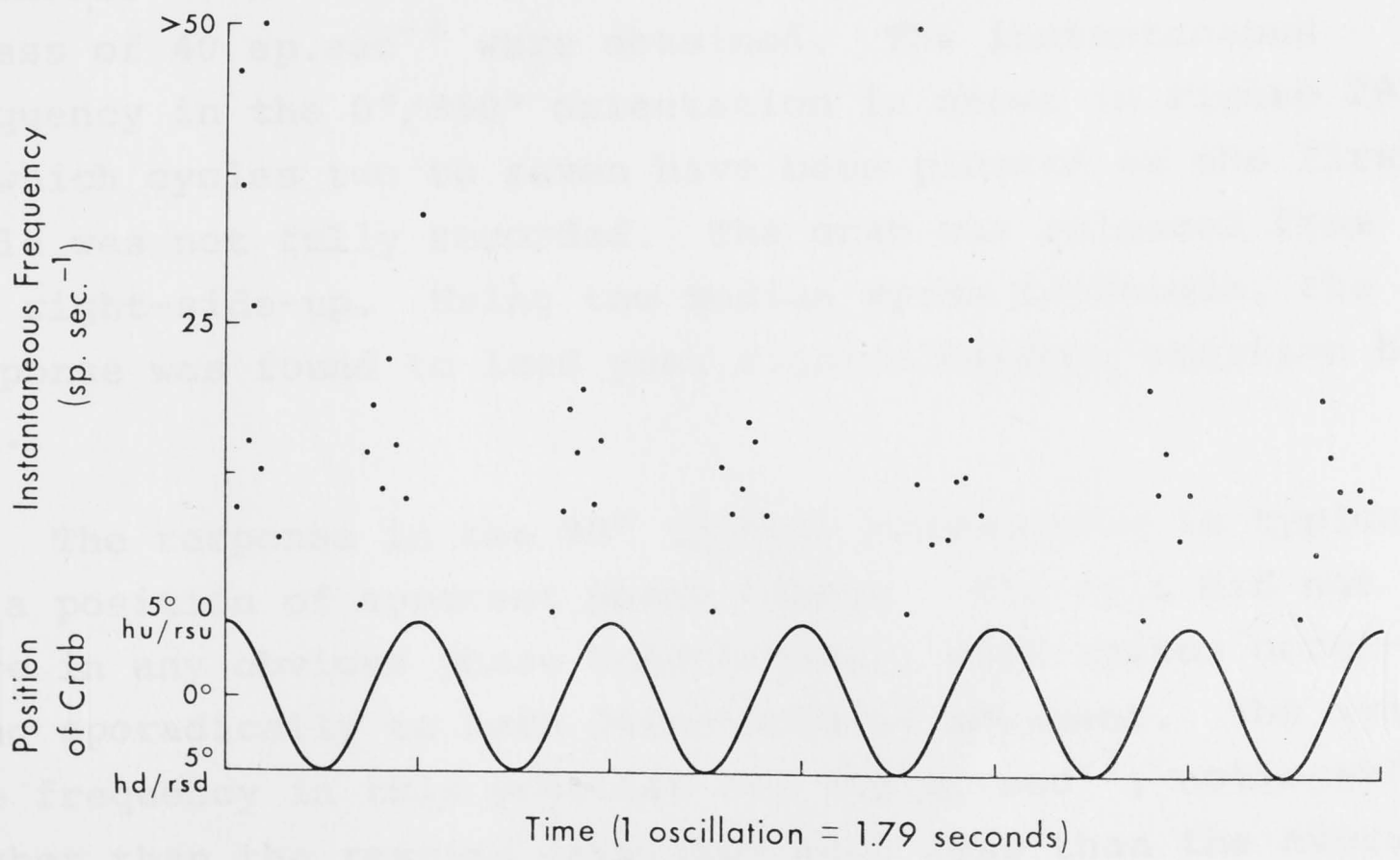
*Figure 26*

The directionality of the response of unit X to sinusoidal oscillation (10° ptp, 0.56 Hz) in different orientations as indicated by silhouettes around the plot. The length of each bar indicates the mean difference in the number of spikes in the two directions of movement, averaging cycles 2 to 6. The scale is 0-10 spikes. The solid arrow shows the position of apparent phase change. The dotted line indicates orientations not sampled.

*Figure 27*

The instantaneous frequency response of unit X to the first 6 cycles of sinusoidal oscillation (10° ptp, 0.56 Hz) after pendulum release.


360°
315°
45°
270°
90°
225°
135°
180°


>50
25
Instantaneous Frequency
(sp. sec.$^{-1}$)
5° 0
hu/rsu
0°
5°
hd/rsd
Position
of Crab
Time (1 oscillation = 1.79 seconds)

The response in the 75° orientation led the peak head-up/right-side-up position by 82° using the median spike technique over the first 6 cycles. This gave a range of 52°-112°, the variability also being evident in Figure 27.

Unit XI

| | |
|---|---|
| Basic discharge rate | 0.5 $sp.sec^{-1}$ |
| Static tilt | no response in pitch or roll planes |
| Continuous oscillation | orientation-dependent, directional, phasic response |
| Maximum directionality | 360°/0°-180° axis |
| Phase in 0°/360° position | 48° lead or peak rsd position (range 38°-56°) |
| Position of apparent phase change | 90° |

Although a complete polar plot was not obtained, the response in orientations at 45° intervals from 0° to 180° gave a very clear indication of the directionality, shown in Figure 28 where the number of spikes per cycle has been plotted. A strongly directional response was elicited by oscillation in the roll plane, the response being to counter clockwise rotation (rsd) during which average frequencies in excess of 40 $sp.sec^{-1}$ were obtained. The instantaneous frequency in the 0°/360° orientation is shown in Figure 29, in which cycles two to seven have been plotted as the first cycle was not fully recorded. The crab was released from 10° right-side-up. Using the median spike technique, the response was found to lead peak right-side-down position by 48°.

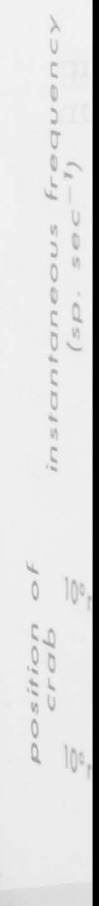
The response in the 90° (pitch) orientation is typical of a position of apparent phase change. The unit did not fire in any obvious phase relationship, with spikes occurring sporadically to both directions of movement. The average frequency in this position was 5.2 $sp.sec^{-1}$, noticeably higher than the resting level but much less than the average

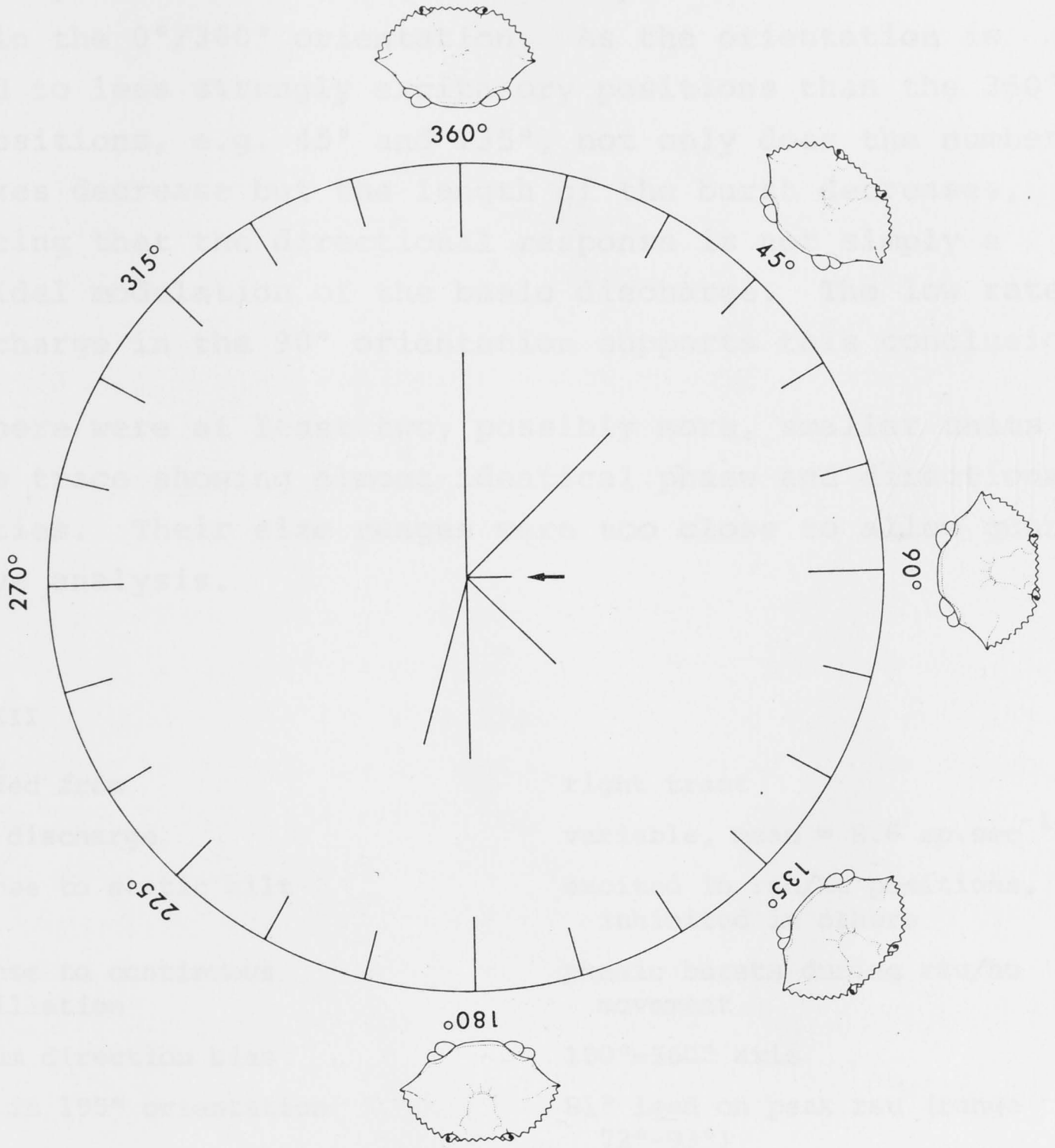
*Figure 28*

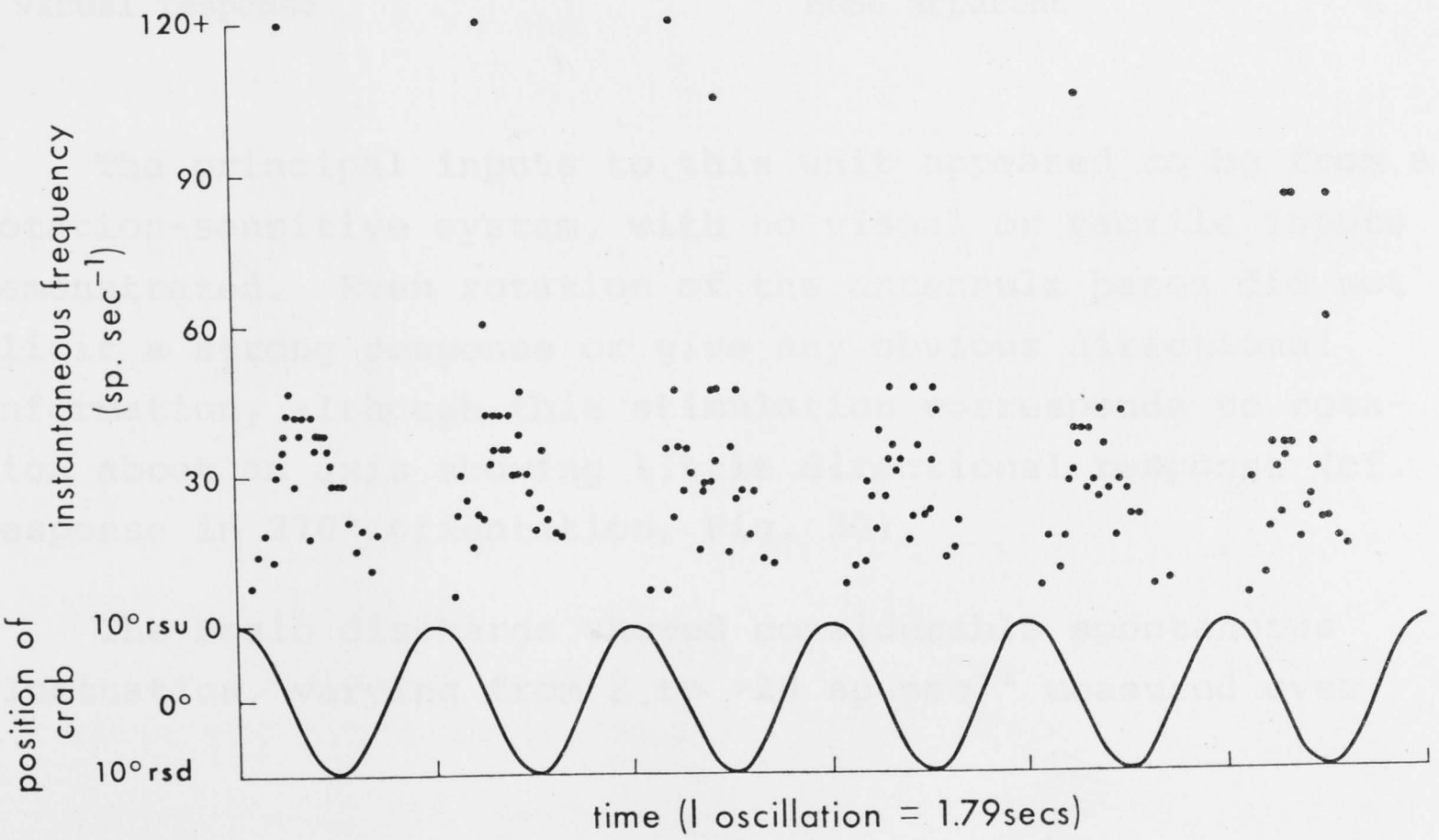
The directionality of the response of unit XI to sinusoidal oscillation (20° ptp, 0.56 Hz) in six different orientations. The arrow indicates the position of apparent phase change. Each bar gives the mean number of spikes in cycles two to seven in a different orientation. The scale is 0-50 spikes.

*Figure 29*

The instantaneous frequency response of unit XI during cycles two to seven of a continuous, sinusoidal oscillation (20° ptp, 0.56 Hz) in the 0/360° orientation.




360°
45°
90°
135°
180°
225°
270°
315°


120+
90
60
30
instantaneous frequency
(sp. sec⁻¹)
10°rsu
0
0°
10°rsd
position of crab
time (1 oscillation = 1.79secs)

of 15.2 sp.sec$^{-1}$ determined over the period of six oscillations in the 0°/360° orientation. As the orientation is changed to less strongly excitatory positions than the 360°-180° positions, e.g. 45° and 135°, not only does the number of spikes decrease but the length of the burst decreases, indicating that the directional response is not simply a sinusoidal modulation of the basic discharge. The low rate of discharge in the 90° orientation supports this conclusion.

There were at least two, possibly more, smaller units in this trace showing almost identical phase and directional properties. Their size ranges were too close to allow quantitative analysis.

Unit XII

| | |
|---|---|
| Recorded from | right tract |
| Basic discharge | variable, mean = 8.6 sp.sec$^{-1}$ |
| Response to static tilt | excited in rsu/hu positions, inhibited in others |
| Response to continuous oscillation | phasic bursts during rsu/hu movement |
| Maximum direction bias | 180°-360° axis |
| Phase in 195° orientation | 81° lead on peak rsu (range 72°-93°) |
| Position of apparent phase change | 285°-300° |
| Visual response | none apparent |

The principal inputs to this unit appeared to be from a rotation-sensitive system, with no visual or tactile inputs demonstrated. Even rotation of the antennule bases did not elicit a strong response or give any obvious directional information, although this stimulation corresponds to rotation about an axis showing little directional response (cf. response in 270° orientation, Fig. 30).

The basic discharge showed considerable spontaneous fluctuation, varying from 2 to >20 sp.sec$^{-1}$ measured over

half-second intervals. However, it was normally close to the mean value of 8.6 $sp.sec^{-1}$ determined over an 8.5 second interval.

The response to maintained tilt of 20° from horizontal was dependent on orientation. The unit was inhibited in right-side-down and head-down positions and excited in the remainder, the position of apparent phase change (285°-300°) also marking the orientation at which a maintained deflection became excitatory rather than inhibitory. The inhibitions were total, and the excitation was weak, reaching only 16 $sp.sec^{-1}$ in the 330° orientation (hd/rsu).

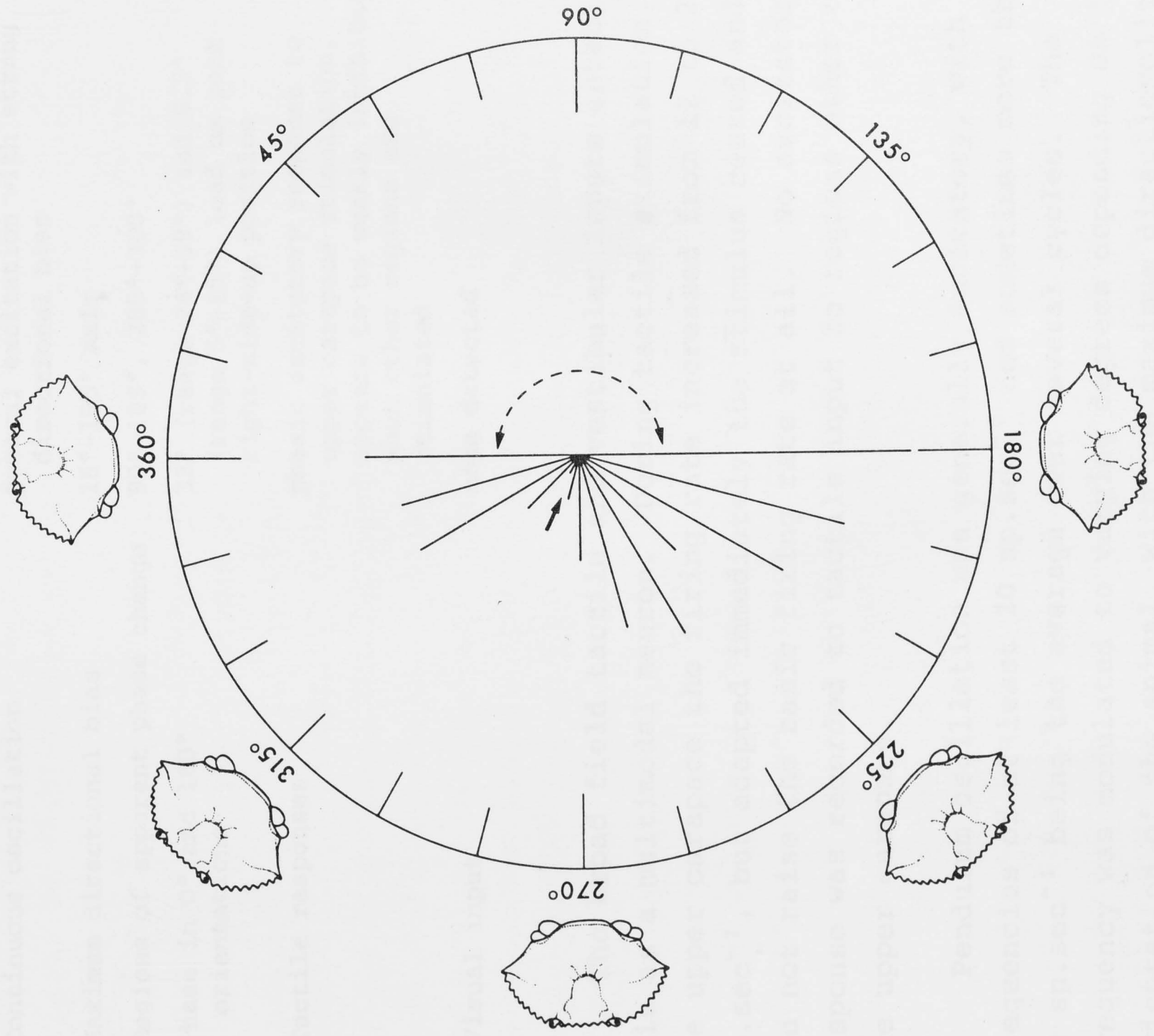
The unit responded to clockwise (rsu) rotation as well as maintained right-side-up position. The response was exceedingly phasic, with more than 70 spikes being elicited to a half-cycle of clockwise rotation in some orientations and complete inhibition occurring during movement in the opposite direction. This first-recorded unit was oscillated at 40° ptp, 0.56 Hz, preventing close comparison to other units. The exceedingly short interspike intervals prevented an accurate analysis of the instantaneous frequency response.

Oscillation at low amplitudes (< 10° ptp) gave a much weaker response (ca. 6 spikes/cycle) in approximately the same phase as for a 40° excursion, which was an 81° lead on peak right-side-up in the 195° orientation. The median spike technique was used.

The position of apparent phase change (285°-300°) indicated a maximally directional response in the 210° and 30° orientations. The rest of the plot does not support this (Fig. 30) despite another measurement confirming the position of apparent phase change as 300°. If this unit had its major rotation-sensitive input from the right vertical canal a position of apparent phase change would be expected at 315° and a maximum response at 225°, where the response is actually relatively weak (Fig. 30). Therefore it seems probable that this is not a canal interneuron.

*Figure 30*

The directionality of the response of unit XIII to sinusoidal oscillation (40° ptp, 0.56 Hz) in different orientations as shown by the outlines of the crabs. The axis of rotation is parallel to the long axis of the page. The solid arrow marks the position of apparent phase change. The dotted line indicates orientations which were not tested. The statistic plotted is the mean difference, during the first 6 cycles, between the numbers of spikes occurring during the two directions of movement in each oscillation. The scale is 0-100 spikes along a radial axis.

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Unit XIII

| | |
|---|---|
| Recorded from | left tract |
| Basic firing rate | slightly variable, 11-14 sp.sec$^{-1}$ |
| Vestibular input | probably mainly right statocyst |
| Static tilt (360° orientation) | very weakly inhibited in 10° right-side-down position, weakly excited in 10° right-side-up position |
| Continuous oscillation | general excitation with strong directional bias |
| Maximum directional bias | 15°-195° axis |
| Regions of apparent phase change | 90°-105°, 285°-300° |
| Phase in 0° and 180° orientations | 32° (range 24°-39°) and 8.5° (range 5°-15°) lead on peak right-side-up position |
| Tactile responses | phasic excitatory response to upper carapace stimulation, appears to be weakly inhibited when other regions are stimulated |
| Visual input | none detected |

The broad field tactile and vestibular inputs establish this as a multimodal neuron. During tactile stimulation of the upper carapace the firing rate increased from 12 to 20 sp.sec$^{-1}$, but adapted immediately the stimulus ceased and did not raise the basic firing rate at all. No excitatory response was recorded to tactile input to regions other than the upper carapace.

Pendulum oscillation was generally excitatory, with frequencies of at least 20 sp.sec$^{-1}$ and sometimes more than 30 sp.sec$^{-1}$ being the average over several cycles. The frequency was modulated to varying degrees depending on the orientation of the animal, with the maximum directionality recorded in the 15° and 195° positions, values also predictable from the positions of apparent phase change (Fig. 31). As the peak of the response occurred close to the maximum deflection of the pendulum (see table, Figs. 33 and 34) the statistic used in plotting Figure 31 is the difference

between the two halves of the cycle divided at the point when the crab is horizontal, e.g. in the 360° position (roll) this would be the right-side-up positions minus the right-side-down. As the response is not perfectly in phase with the oscillation (table above, Figures 33 and 34) this method slightly underestimates the directionality. The positions of maximum directionality, 15° and 195°, are close to pure roll (0/360° and 180°) and the unit fires most strongly on right-side-down.

The origin of rotational sensitivity is not clear. Tilting the antennule bases was only weakly excitatory with no clear directionality, but as this stimulation corresponds approximately to rotation in a null position the result is inconclusive. Yawing the animal was excitatory but with no obvious directional discrimination. Opening the right statocyst abolished the directional response in the roll position (360°) and the overall firing rate was lower than for any other oscillation. As the statolith is situated in the vertical canal its responses should have been affected by this procedure, but to an unknown degree. Canal function would have been drastically disrupted.

The polar plot shows not only strong responses for the right canal optimal positions (45° and 225°) but also for one of the canal null positions (135°) although the response is weak in the other (315°). For this reason it seems that a statolith input is the most likely.

The phase difference between peak right-side-down position and peak response varies with starting position (table above, Figs. 33 and 34). Where the initial position was 10° right-side-up (360° position) the peak frequency of the response led the position peak by 32°. This lead reduced to 8.5° when the starting position was 10° right-side-down (180°). This difference is one reason Figure 31 is not symmetrical about the axis 105-285°, as in the 105→285° section of the plot the excitatory and inhibitory parts of the

cycle fit the sample bins more precisely than they do in the other part of the plot.

A further reason for the imbalance is the effect of the initial cycle. If the initial direction of movement is excitatory then the difference between the two halves of the first cycle is considerably greater than the average for the subsequent oscillations. Conversely if the initial direction is inhibitory the difference between the two halves is generally less than in subsequent oscillations. The positions where the initial movement is excitatory are from 105° to 285°. The initial cycle differences are shown in Figure 32, where the dotted arrows are the positions of apparent phase change as determined over an average of 6 cycles in Figure 31, and the solid arrows are the positions of apparent phase change when only the first cycle is considered. The pair of arrows which do not coincide indicate a position where, in the first oscillation, the first half-cycle gives a stronger response than the second, but in the following oscillations the second half-cycle is stronger. The asymmetry mentioned above in relation to Figure 31 is very obvious here, but there is no fundamental difference in the alignment of the two plots.

Other plots made using different statistics obtained from the same recordings showed only superficial differences from Figure 31.

The general excitatory effect of motion was further demonstrated during the testing for static tilt. These tests were made in the 360° (roll) position, which is very close to the position of optimum directionality. The animal was tilted by hand from the horizontal to either 10° right-side-up or 10° right-side-down, and maintained in this section for several seconds.

10° right-side-down

| | |
|---|---|
| initial (horizontal) frequency | 13.5 $sp.sec^{-1}$ |
| frequency during rotation | 15.0 $sp.sec^{-1}$ |
| frequency ($sp.sec^{-1}$) in 1 sec intervals in maintained position 10° rsd | 10 12 9 8 8 8 9 12 9 13<br>15 10 14 |

10° right-side-up

| | |
|---|---|
| frequency during rotation (1 sec intervals) | 28 36 36 |
| frequency ($sp.sec^{-1}$) in 1 sec intervals at maintained position -10° rsu | 30 30 27 40 28 15 16 15<br>14 15 15 15 17 |
| frequency ($sp.sec^{-1}$) in 1 sec intervals in maintained position — horizontal | 22 24 26 16 |

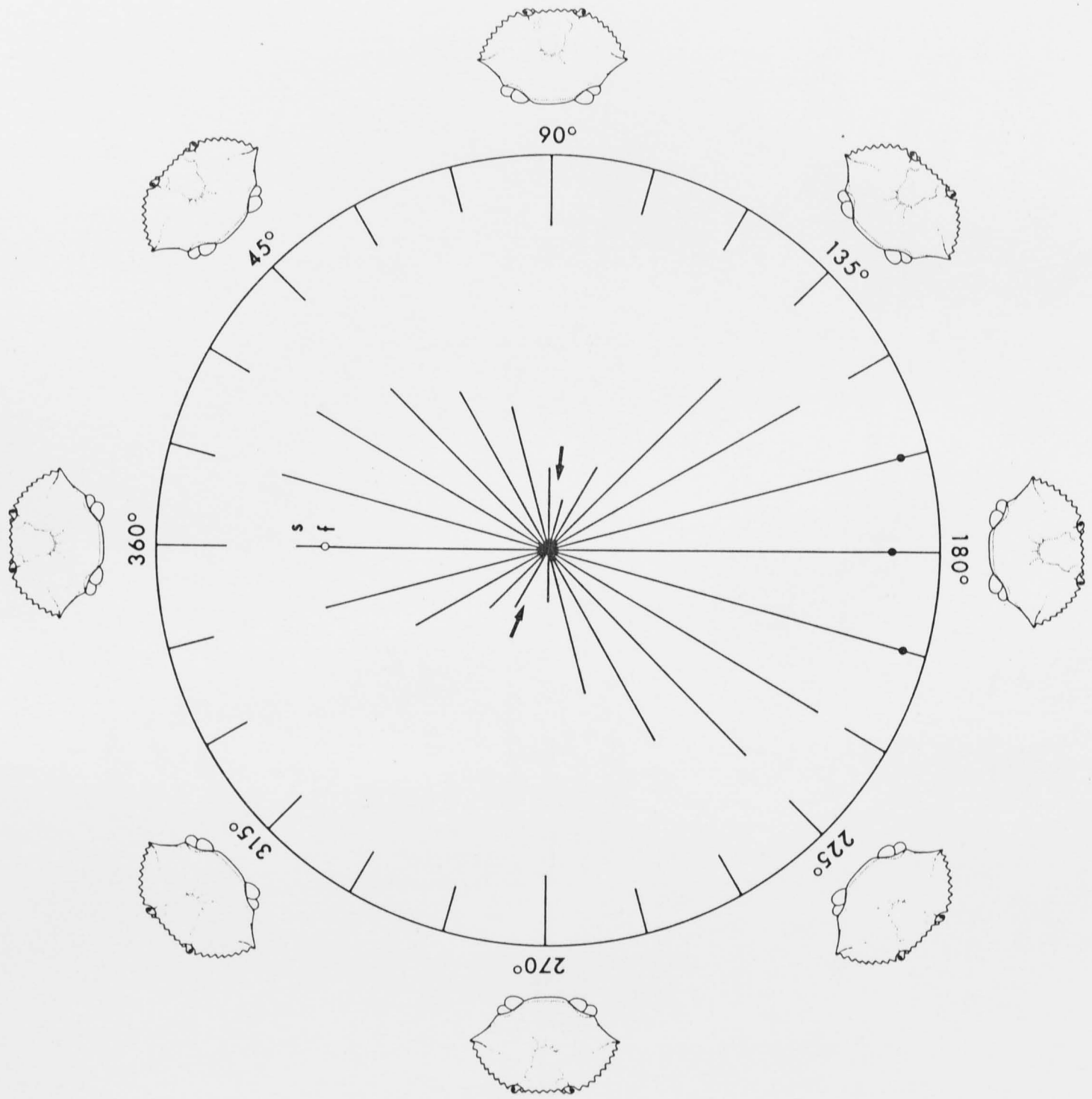
The increased firing rate during movement in the cw (rsu) direction and the high initial discharge in the 10° rsu maintained position support the earlier assessment of phase relationships with respect to position. The adaptation occurring after 5 seconds of maintained rsu position suggests that up to this time the response might have been attributable to a more general excitation caused by the movement just completed. This would also explain the initially higher rate in the horizontal position at the completion of the experiment. Similarly, movement in the ccw (rsd) direction, that is, to the "inhibitory" position of the dynamic tests, appears to inhibit the unit for several seconds before it reaches a firing rate similar to that in the 10° rsu position. Nevertheless over a short period of time the unit appears to show an excitatory response to maintained tilt 10° rsu, and a slight inhibition to maintained tilt 10° rsd, in approximate agreement with what might be expected from the experiments with the oscillating pendulum.

*Figure 31*

The directionality of the response of unit XIII to sinusoidal oscillation (20° ptp. 0.56 Hz) in different orientations indicated by the silhouettes around the periphery. The values plotted are the mean differences in spike number between the two halves of the first six cycles. The cycle has been divided at the point at which the pendulum is vertical. The axis of oscillation is parallel to the long axis of the page. The solid arrows indicate the positions of apparent phase change. The open circle in the 360° orientation indicates the value at the completion of the plot, which was made in the sense 0°-360°. The other open circles represent the values in those orientations. Scale 0-25 spikes.

*Figure 32*

The directionality of the response of unit XIII to sinusoidal oscillation. The conditions are the same as in Figure 31, but only the first cycle has been plotted. The solid arrows indicate the positions of apparent phase change. The dotted arrows indicate the positions of apparent phase change in Figure 31. Scale 0-25 spikes.



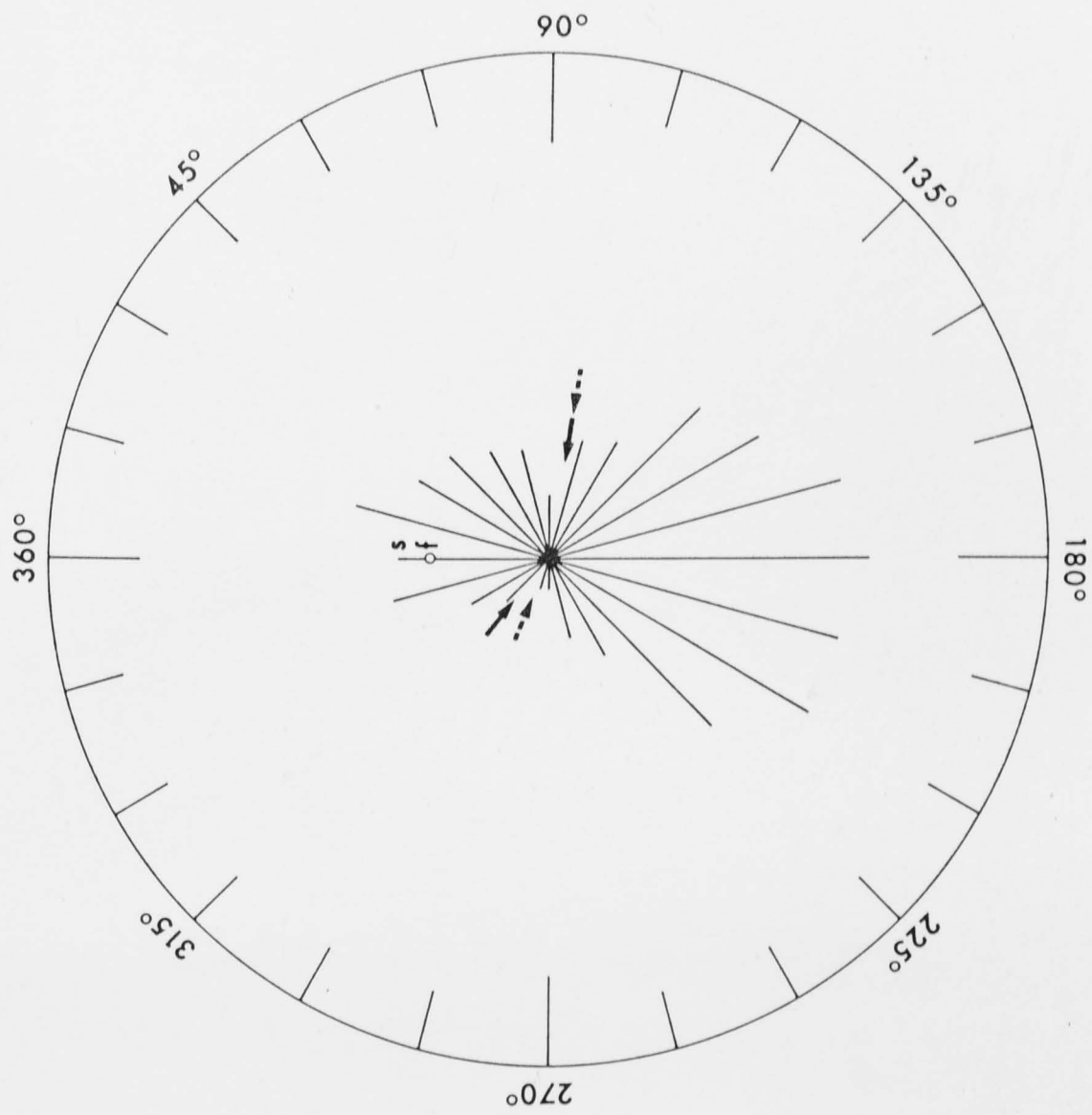




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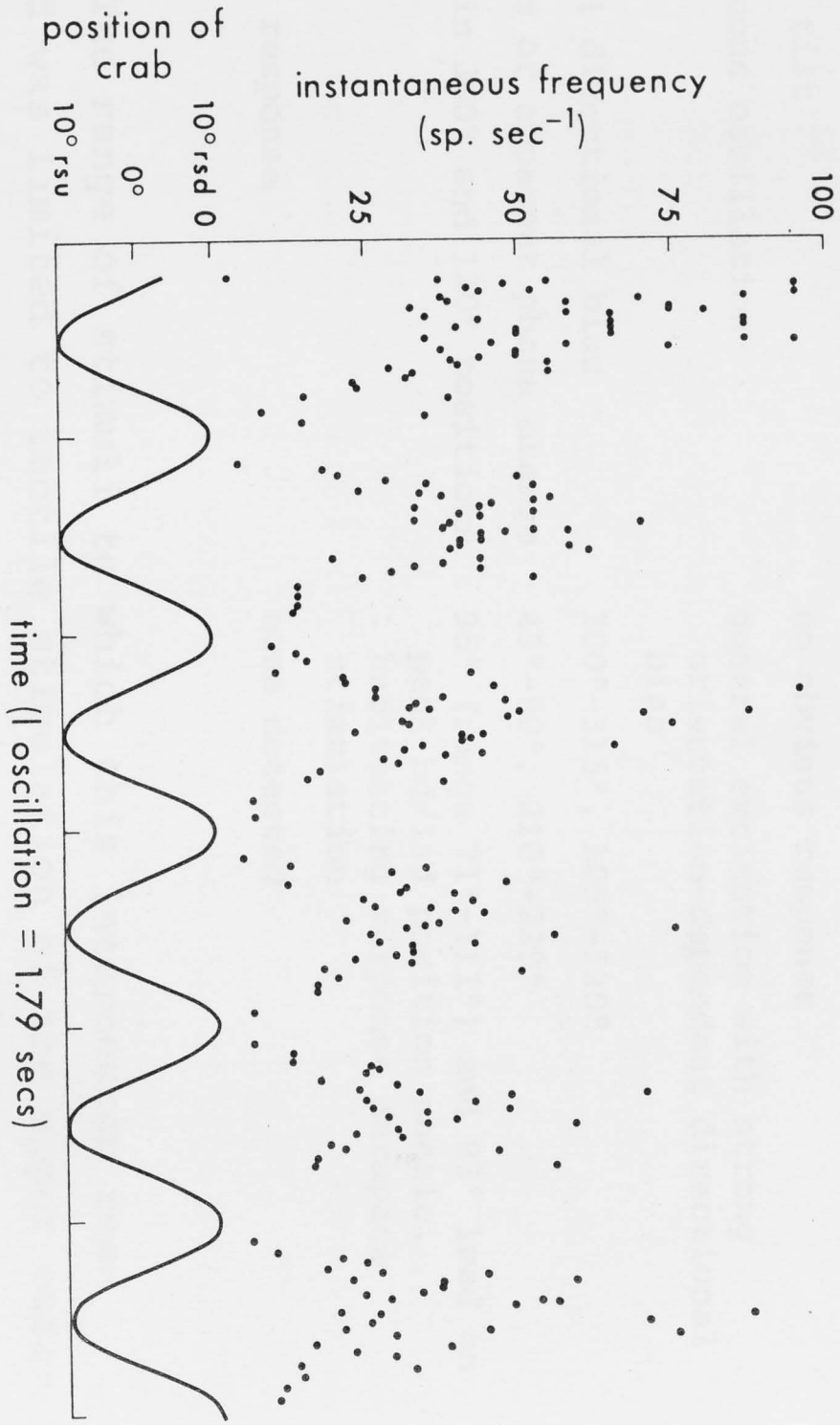
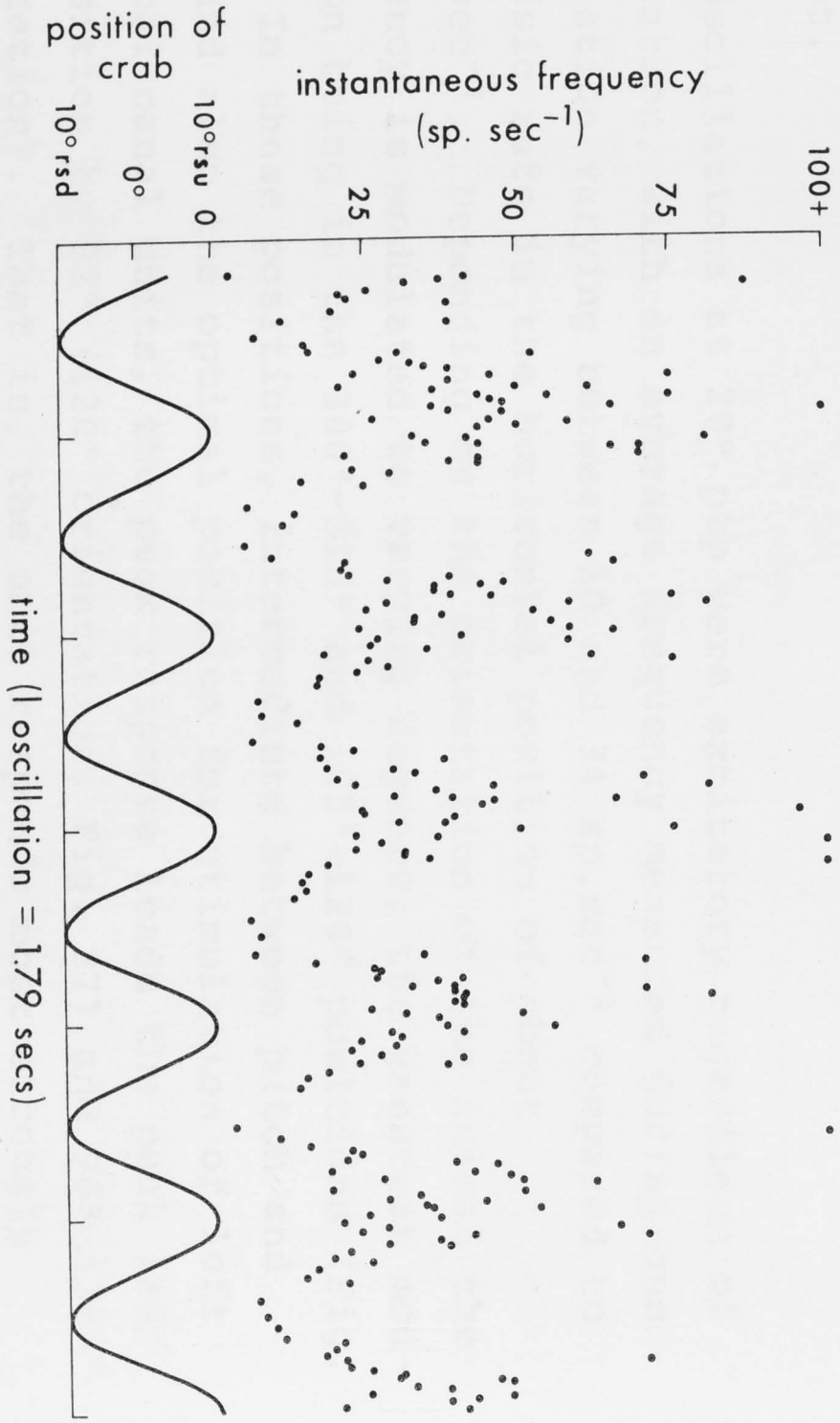
*Figure 33*

The instantaneous frequency response of unit XIII to the first six cycles of sinusoidal oscillation (20° ptp, 0.56 Hz) in the 180° orientation. The recording does not start immediately the pendulum is released.

*Figure 34*

The instantaneous frequency response of unit XIII to the first six cycles of sinusoidal oscillation (20° ptp, 0.56 Hz) in the 260° orientation. The recording does not start immediately the pendulum is released.


position of crab
10° rsd
0°
10° rsu 0
instantaneous frequency
(sp. sec$^{-1}$)
25
50
75
100+
time (1 oscillation = 1.79 secs)


position of crab
10° rsu
0°
10° rsd 0
instantaneous frequency
(sp. sec$^{-1}$)
25
50
75
100
time (1 oscillation = 1.79 secs)

Unit XIV

| | |
|---|---|
| Recorded from | left tract |
| Basic firing rate | 5.9 sp.sec$^{-1}$ (average over 9 seconds) |
| Vestibular input | left (ipsilateral) statocyst |
| Static tilt | no obvious response |
| Continuous oscillation | general excitation with strong orientation-dependent directional bias |
| Maximal directional bias | 300°-315°, 105°-120° |
| Regions of apparent phase change | 45°-60°, 210°-225° |
| Phase in 300° and 120° positions | 96° (range 71°-111°) and 92° lead on peak hd/lsd position phasic, habituating response carapace stimulation |
| Visual response | none detected |

The range of stimuli to which this interneuron responded was limited to tactile stimulation of the upper carapace, rotation of the right antennule base, which appeared to be a weakly-excitatory tactile input, and rotation of the whole animal.

The response to static tilt was variable with the only obvious generalisation being that movement to the tilted position was excitatory but the actual maintained position was not.

Oscillations at 20° ptp were excitatory regardless of orientation, with an average frequency measured during one oscillation varying between 20 and 34 sp.sec$^{-1}$ compared to the basic rate in the horizontal position of about 6 sp.sec$^{-1}$. Depending on the orientation of the animal the frequency is modulated to varying degrees, the greatest modulation being in the 300°-315° and 105°-120° positions (Fig. 35). In these positions, intermediate between pitch and roll and also the optimal position for stimulation of left vertical canal units, the peak response leads the peak lsd/hd position by 92° (120° orientation, Fig. 37) and 96° (300° orientation). That is, the unit responds most strongly

about the peak velocity in the head-down/left-side-down sense, and the phase lead is slightly greater when the starting position is head-up/left-side-up than when it is hd/lsd. The phase relationship varies from cycle to cycle and the difference between the values in the two orientations is negligible. The unit also shows obvious brief sporadic increases and decreases in frequency with no obvious cause. These and any more subtle changes would contribute to variations in phase and in the magnitude of the responses in different orientations in the polar plots.

The contralateral (right) antennule was removed without losing the unit, and Figure 36 shows the polar plot determined under exactly the same conditions as Figure 35. The statistic plotted is the mean, over 6 cycles, of the differences in spike number between two halves of each oscillation divided at the point the direction of movement changes, or, more simply the first half cycle is measured from when the pendulum is released. The similarity between the two plots is very close and is even reflected in certain apparent irregularities in each plot, for example the response in the 0° (s), the 135° and the 270° position. In Figure 36 one position of apparent phase change (210°-225°) has shifted, and is now exactly 180° from the other. After removal of the right antennule base the basic firing rate was 5.5 $sp.sec^{-1}$ averaged over 12 seconds, very close to the pre-ablation level of 5.9 $sp.sec^{-1}$. Further, the average frequency during rotation is also approximately the same as before the right antennule base was removed, varying from 14 to 32 $sp.sec^{-1}$ averaged over 6 cycles (10 seconds) but seldom reaching these extremes. From this it seems justifiable to conclude that neither the directional discrimination nor the level of excitability of this unit are dependent on input from the contralateral (right) statocyst.

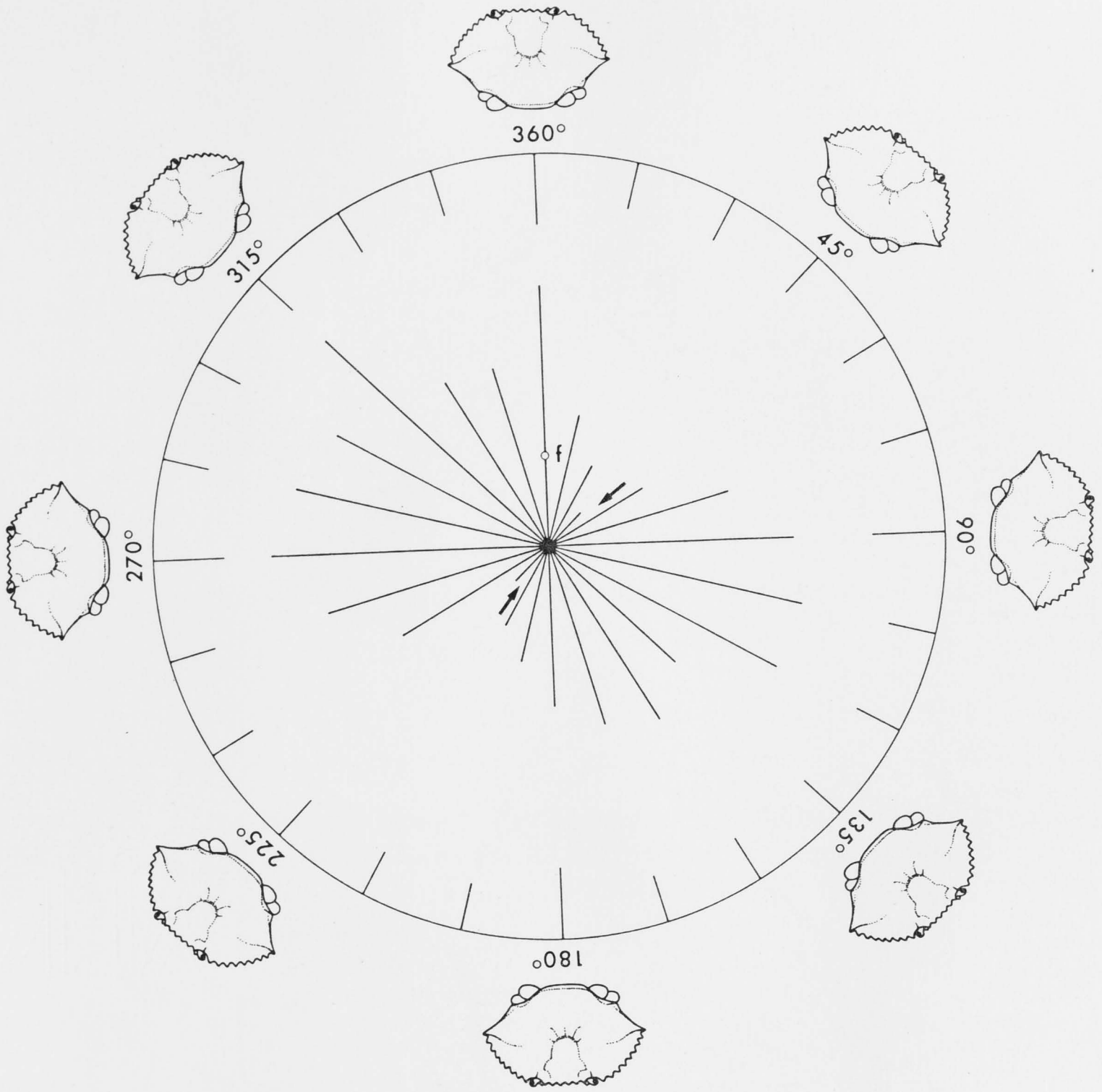
This unit has several similarities to other units in this study which appear to have statolith input, but this particular unit also has its greatest discrimination of

*Figure 35*

The directionality of the response of unit XIV to sinusoidal oscillation (20° ptp, 0.56 Hz) in different orientations, indicated by the silhouettes around the graph. The length of the line indicates the mean difference in spike number in the two directions of movement in each orientation. Scale 0-25 spikes. The open circle in the 360° orientation is the value determined at the conclusion of the plot. The solid arrows indicate the positions of apparent phase change.

*Figure 36*

The effect of removing the right antennule base on the directionality of the response of unit XIV to sinusoidal oscillation. The details are identical to those in Figure 35.


360°
45°
90°
135°
180°
225°
270°
315°
f

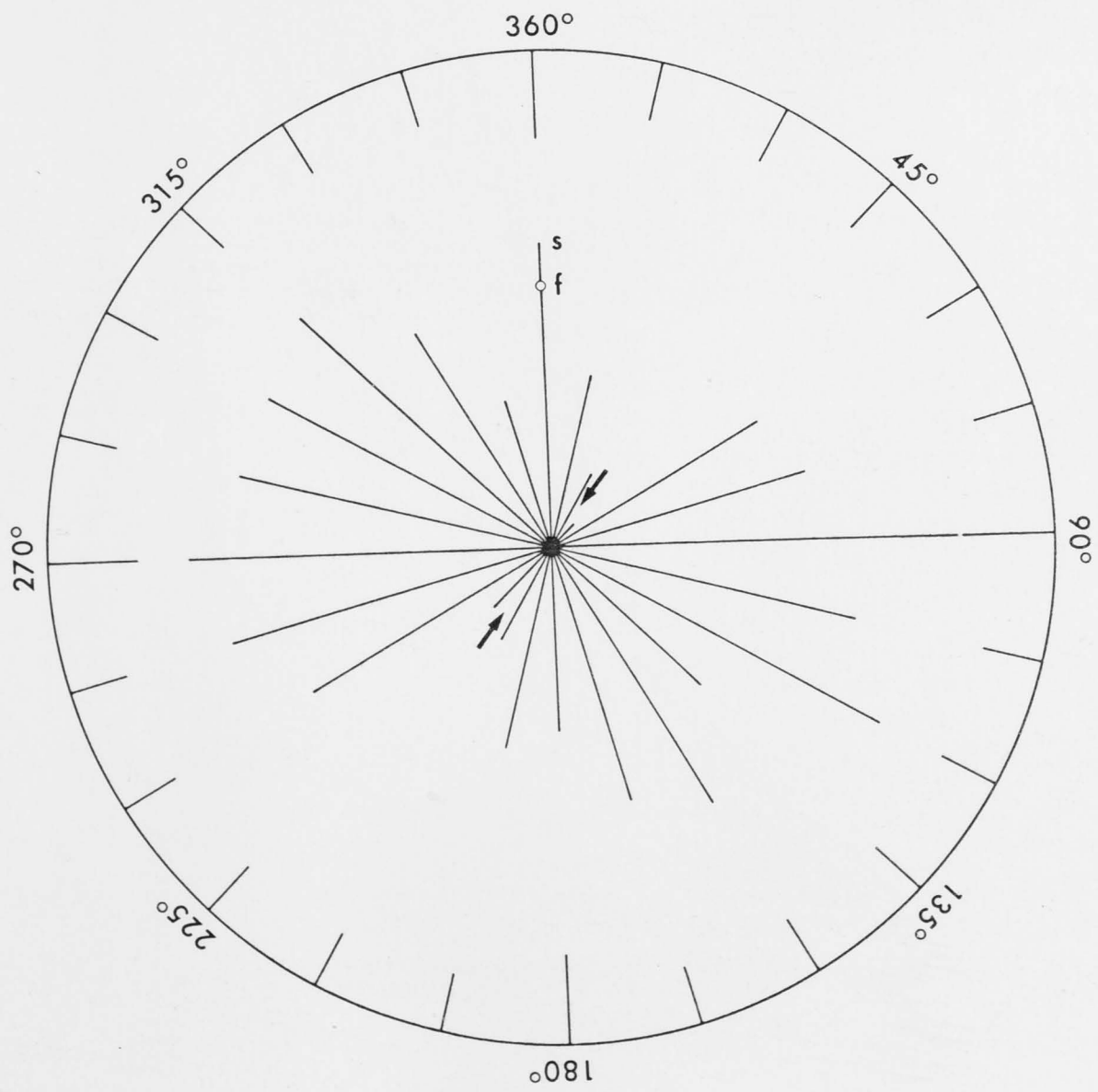
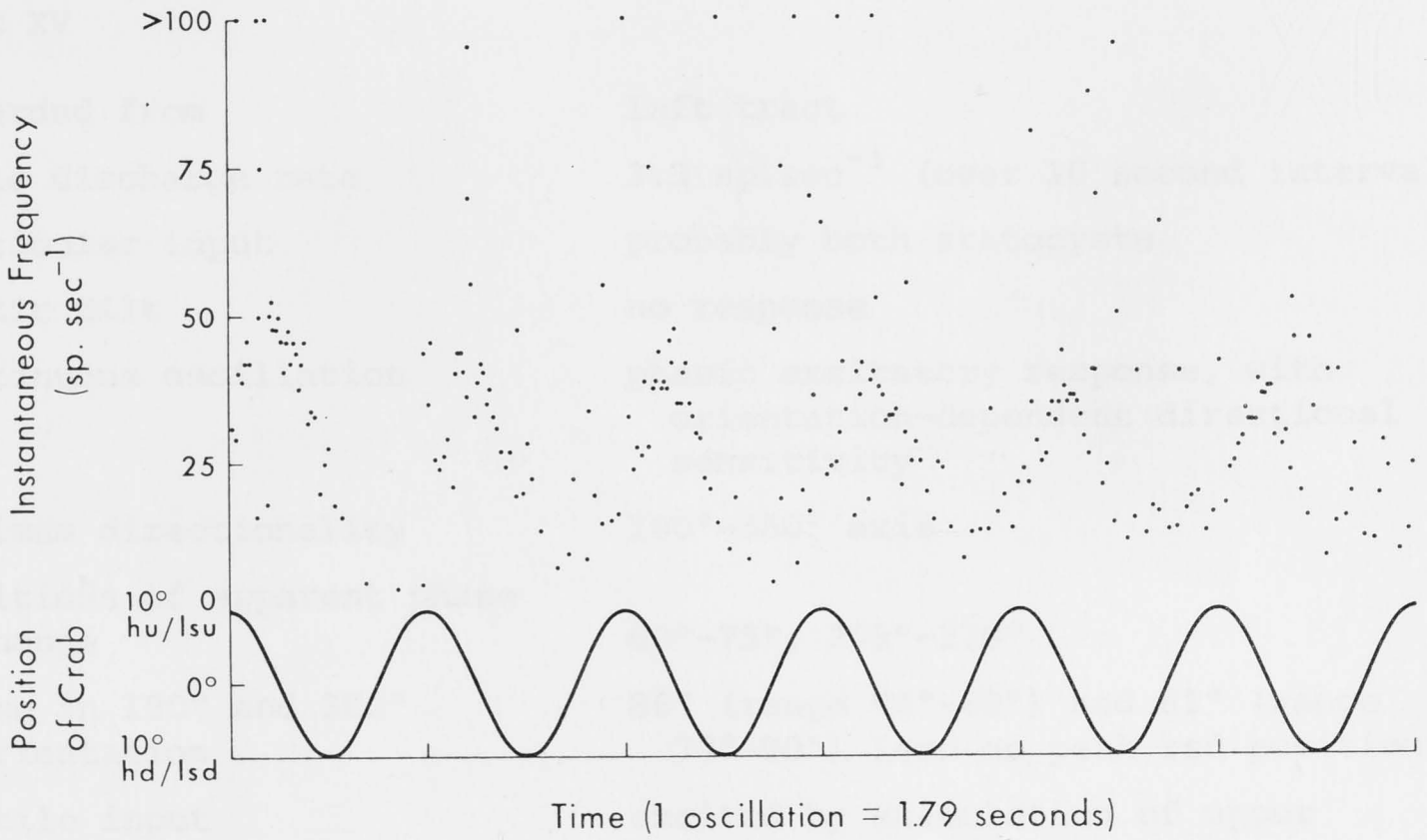

360°
45°
90°
135°
180°
225°
270°
315°
s
f

*Figure 37*

The instantaneous frequency response of unit XIV to the first six cycles of sinusoidal oscillation (20° ptp, 0.56 Hz) in the 120° orientation.


>100
75
50
25
0
Instantaneous Frequency (sp. sec$^{-1}$)
Position of Crab
10° hu/lsu
0°
10° hd/lsd
Time (1 oscillation = 1.79 seconds)

direction close to the optimal position for stimulation of units in the left vertical canal. The work of Fraser and Sandeman (1975) and Janse and Sandeman (in preparation) has shown that the phase relationship between stimulus and response of thread hair units is very sensitive to starting position of the animal, even for relatively small differences. Here the difference in starting positions is much greater (10° hd/lsd or 10° hu/lsu = 20° difference) than the 13° total displacement of Fraser and Sandeman (1975) yet there is virtually no change in the phase relationship, suggesting that this is also a statolith unit. The free hook hairs, the third possible input, have not been shown to respond to low frequency oscillations in the intact statocyst.

Unit XV

| | |
|---|---|
| Recorded from | left tract |
| Basic discharge rate | 3.3 $sp.sec^{-1}$ (over 10 second interval) |
| Vestibular input | probably both statocysts |
| Static tilt | no response |
| Continuous oscillation | phasic excitatory response, with orientation-dependent directional sensitivity |
| Maximum directionality | 180°-360° axis |
| Positions of apparent phase change | 60°-75°, 255°-270° |
| Phase in 180° and 360° orientation | 86° (range 84°-89°) and 81° (range 77°-90°) lead on peak rsd position |
| Tactile input | excited by stimulation of upper carapace leg stumps, and by antennule base rotation |
| Visual input | gating — intensified rotational response. No other effect observed |

Unit XV is clearly a multi-modal interneuron, responding to rotational and tactile stimuli; and showing visually mediated modifications to the rotational response.

Any movement is excitatory, with the exception of rapid

counter-clockwise roll, which is inhibitory. The directionaltiy of the rotational response is shown in Figure 38. The length of the bar in each position indicates the mean number of spikes in the most excitatory 90° interval in each cycle, averaging six cycles in each orientation. This gives an average frequency during the most excitatory part of each cycle and tends to underestimate the directionality compared to the methods used for some other units. The orientations showing greatest sensitivity to rotation lie close to the 360°-180° axis where the unit fires to counter-clockwise rotation. In these positions the unit is completely inhibited in the non-excitatory direction (Figs. 39 and 40). The positions of apparent phase change (60°-75°, 255°-270°) are close to being at right angles to the 360°-180° axis, although the positions are themselves not 180° apart. The average frequency in the 270° orientation was 8 $sp.sec^{-1}$, rising to 10.7 $sp.sec^{-1}$ in the 75° orientation, where firing was more or less continuous but still showing peaks about the stimulus velocity peak. The average frequency in the 180° orientation was 18 $sp.sec^{-1}$, but reached 49 $sp.sec^{-1}$ during a quarter-cycle period. In the 360° orientation the mean frequency was 22 $sp.sec^{-1}$ but reached a value of 69 $sp.sec^{-1}$ over a quarter-cycle interval. After the polar plot was completed the resting discharge in the horizontal position was 8 $sp.sec^{-1}$. From this it appears that the response is not simply a sinusoidal modulation of a constant, relatively high discharge.

Unit XV fires with the highest frequency when the animal is oscillated in approximately the roll plane. Units apparently from the canal but firing only in the roll plane and not to pitch have been observed (unpublished observations) but these units showed maximal responses when in the optimum position for canal stimulation. Vertebrate canal units have similarly been shown to respond maximally when rotated in the plane of the canal (Lowenstein 1974). This suggests that unit XV is not a canal unit although the

strongest responses do occur in the 315° and 330° orientations, close to the optimal orientation for left vertical canal stimulation. However there was no correspondingly high response in the 135° and 150° orientations (the other left canal optimum) and the shape of the plot did not indicate that the 315° and 330° orientations were on a canal maximum as the responses in the adjacent orientations were weak (Fig. 38). The values at 315° and 330° are probably attributable to changes in excitability discussed below.

Further evidence that this is not a canal unit comes from the phase relationship of the response to the stimulus. In fibre 5 of the circumoesophageal connective of *Scylla* (a unit with known thread hair input) (Fraser and Sandeman 1975) the first head-up rotation gives no response if the animal is released from a head-down position, but if the crab is initially head-up the first head-up rotation is excitatory. Other experiments rule out the apparently obvious conclusion that the response is to head-down rotation but lags peak head-down position by 90°, and the effect appears attributable to the initial position. Janse and Sandeman (in preparation) have also shown a marked dependence of thread hair mediated responses upcn initial position of the body with respect to gravity. Figures 39 and 40 show the instantaneous frequency plots for oscillation in the 360° and 180° orientations. That is, for roll starting from right-side-up and then left-side-up. Apart from the apparent 180° phase shift introduced by the mechanics of the apparatus there is a mean phase shift of about 5° between the two conditions, with the response leading position by slightly more in the 180° orientation (table above). The value of 5° was obtained both from the plots and by the median spike method. This difference is probably not significant as the variation from cycle to cycle was greater than ±5°. It must be remembered that for accurate determination of phase shifts caused by the positioning of the body before release the animal should be left in the required position for several minutes to allow hair positions to reach

equilibrium with gravitational forces. This unit probably was not fully equilibrated to each position but the minimal phase shift supports the contention that unit XV does not have thread hair input. The other class of canal unit, the free hook hairs, have not been shown to respond to low frequencies of oscillation in the intact system.

The peak response in the 180° and 360° orientations led the peak right-side-down position, by 86° and 81° respectively, when oscillated 20° ptp at 0.56 Hz. This is close to the velocity peak in the counter-clockwise direction.

As with many others in this study, unit XV showed considerable variability in its response to identical stimuli, a phenomenon referred to as a "change in central excitability". This occurs here as a change in the resting level, a change in the response during a few cycles, or a prolonged change, such as has probably occurred at 315° and 330° in Figure 38. Changes in both the basic firing rate and the response to oscillation could be induced by other modalities. Stimulation of the leg stumps with a fine brush caused a discharge of 37 $sp.sec^{-1}$ during the four seconds of stimulation and the level then rose to 50 $sp.sec^{-1}$ for about three seconds before decreasing slowly. In contrast the same treatment applied to the upper carapace gave only a weak phasic burst which ceased abruptly when the stimulus ceased.

Oscillation of the antennule bases by hand also caused a high sustained level of discharge which may result either from intense stimulation of the statocyst or from tactile or proprioceptors in the antennule base. No rotational response was observed, but the antennule bases were oscillated in approximately the pitch plane where unit XV showed very little directional selectivity.

The polar plot and other tests were carried out in a dimly-lit room which resulted in the same rotational responses as when the eyes were blacked out. When the room light was on and the eyes uncovered the average frequency of

the most excitatory quarter-cycle during roll was increased by 28% (17 sp.sec$^{-1}$). No visual responses were elicited by changes in light intensity or by localized or broad-field movements in the visual field. The phase relationship under conditions of bright illumination was identical to that in the dark. It appears that a general relatively-intense illumination has acted as a gating stimulus rather than as an additional, independent, rotation-sensitive input.

In contrast to the general finding in this study, the response during the first cycle of oscillation was often rather weaker than on subsequent cycles. As neither the polar plot nor rotation of the antennular bases indicated the origin of the direction-sensitive rotation response the right antennule base was arbitrarily chosen for removal. The resting level was reduced to a level that varied from less than 0.2 sp.sec$^{-1}$ up to 2 sp.sec$^{-1}$, but was mainly in the lower part of this range. Trauma of any sort normally raises the resting level, which suggests that in this instance part of the input maintaining the normal resting level was removed.

Directional sensitivity to rotation was still present, but the form of the response was changed drastically. The responses were invariably weaker than in the intact animal, with only one run giving more than 10 spikes per cycle. The number was also variable as the data for one run in the 90° (pitch) orientation show:

| | |
|---|---|
| No. of spikes/cycle | 5, 0, 2, 3, 2, 3, 4, 8, 10, 8, 8, 17, 20, 16, 10, 5, 2, 1, 1, 0, 3, 3, 5 |

Another run in the 270° (pitch) orientation did not respond for at least the first six cycles, but then began to fire. All responses were approximately in phase with the peak of velocity in the head-down or right-side-down directions, with long periods of inhibition in between. Although it was difficult to determine phase relationships precisely with only a small number of spikes the median spike method gave a

phase lead of 94° (range 80°-118°) on peak right-side-down position in the 360° roll orientation. Considering the other alterations in the response this compares well with the value of 81° obtained in the unoperated animal. These data clearly suggest that the left statocyst provides at least some of the discrimination of the direction of rotation. No polar plot could be made because of the variability of the response in any given orientation and it appears that the right statocyst must be intact for the unit to function with its normal sensitivity.

Listed below are the number of spikes per cycle for two consecutive runs made in the 90° (pitch) orientation. The first was made with the room light off (this did not give absolute darkness), and the second with the light on.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Light off | 7 | 7 | 4 | 3 | 2 | 0 | 0 | 0 | 0 | 0 |
| Light on | 15 | 18 | 21 | 21 | 23 | 26 | 27 | 21 | 24 | 25 |

The response in the light on condition led peak head-down position by 105°, whereas the phase lead from a more strongly responding part of the 90° run listed above was 108°. As in the intact animal, covering the eyes did not diminish the response to a lower level than in the darkened room. Therefore the simplest explanation again is that strong visual input increases the sensitivity of the system without being the principal movement detector.

The left (ipsilateral) antennule was removed. With the eyes covered the unit fired twice in 22.5 seconds (0.09 $sp.sec^{-1}$). Stimulation of the leg bases with a paintbrush evoked a discharge of 36 $sp.sec^{-1}$, rising to 50 $sp.sec^{-1}$ as discussed above. Oscillating the animal in the roll plane elicited three bursts approximately in phase with peak right-side-down position, but any phase relationship was then lost, although the unit continued to fire at a frequency of 3 $sp.sec^{-1}$ or greater. Uncovering the eyes and turning on the room light did not change the response to rotation except that the firing rate did not drop below

*Figure 38*

The directionality of the response of unit XV to sinusoidal oscillations (20° ptp, 0.56 Hz) in different orientations, indicated by the crab silhouettes around the margin. The axis of oscillation is parallel to the long axis of the page. The crab was released from head-up at 90°, right-side-up at 360°. The solid arrows indicate the positions of apparent phase change. The open circle shows the value in orientation 270° at the beginning of the plot. The length of the bar gives the mean number of spikes in a 90° interval during each cycle. The scale is 0-50 spikes along a radial axis.

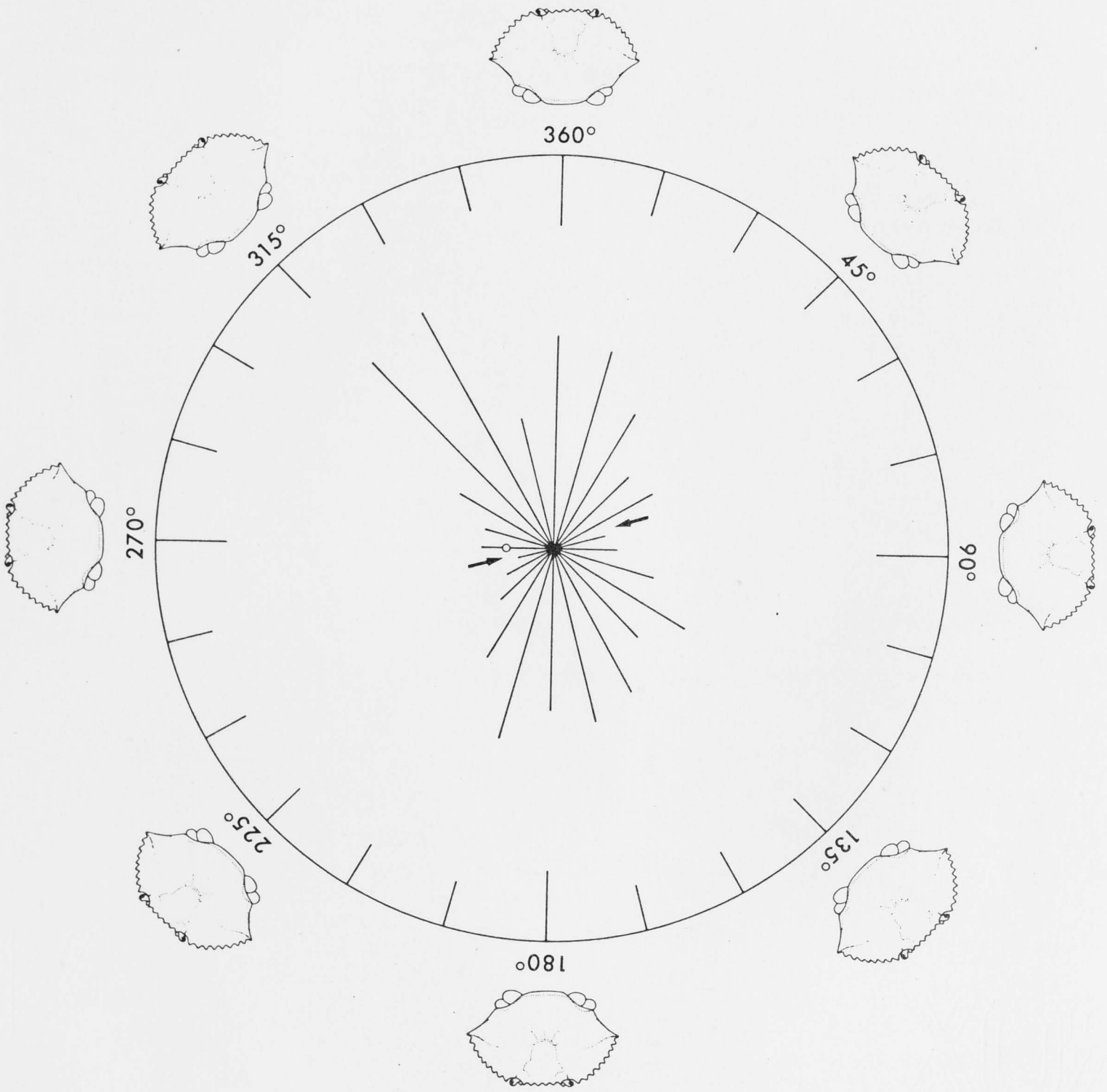
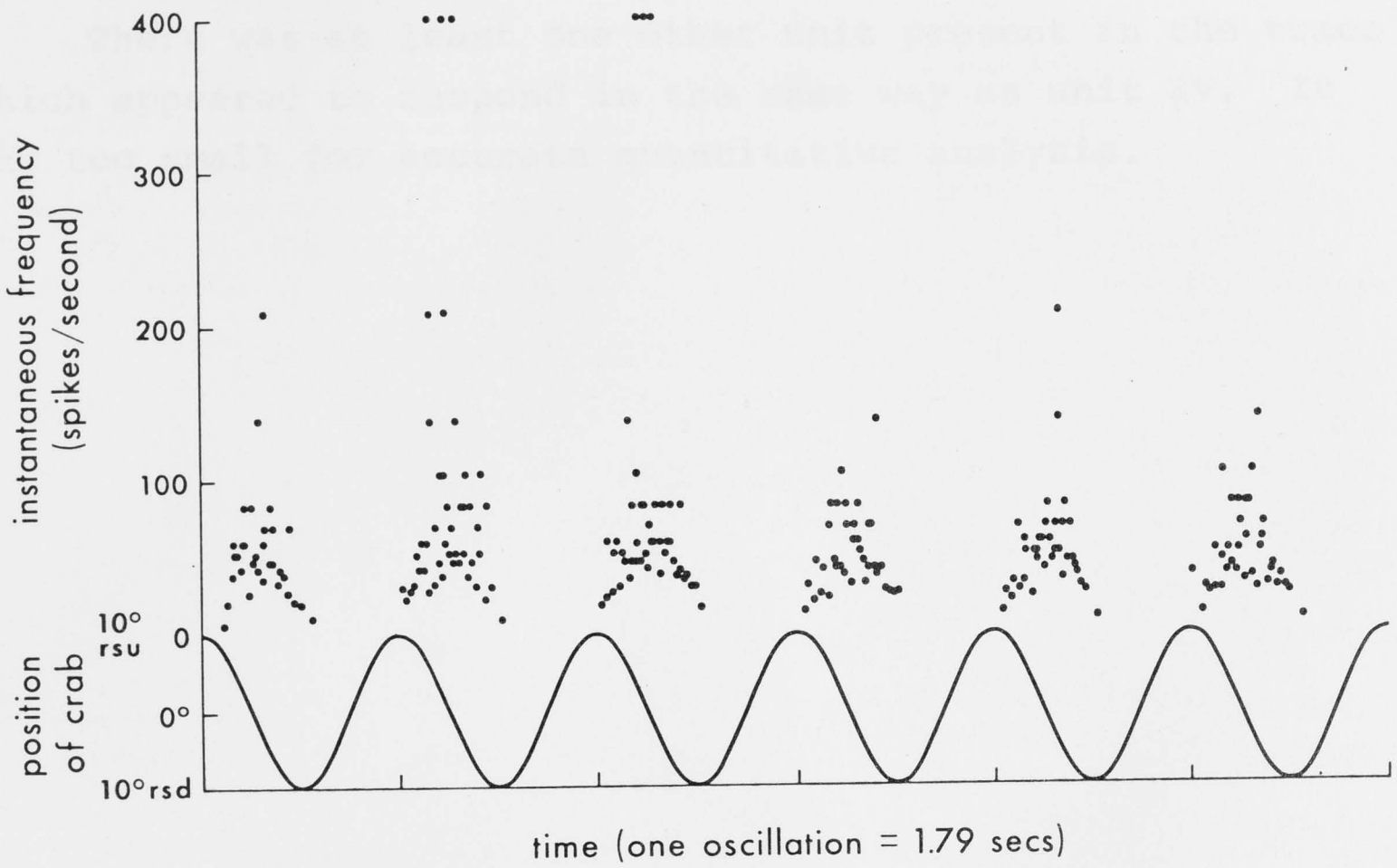

360°
315°
45°
270°
90°
225°
135°
180°

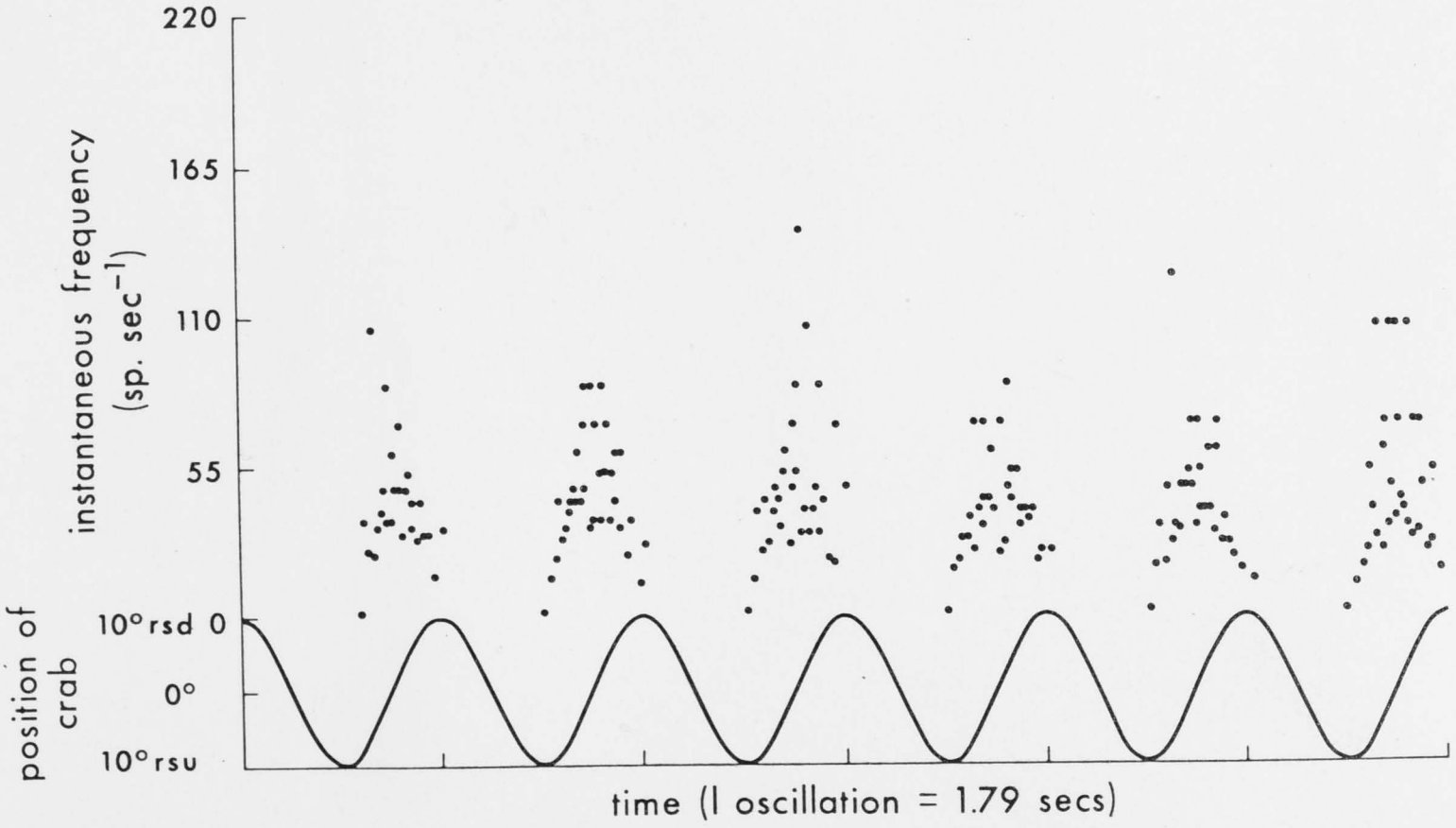
*Figure 39*

The instantaneous frequency response of unit XV during the first 6 cycles of sinusoidal oscillation (20° ptp, 0.56 Hz) in the 360° orientation.


*Figure 40*

The response of unit XV during the first 6 cycles of sinusoidal oscillation (20° ptp, 0.56 Hz) in the 180° orientation.


400
300
200
100
instantaneous frequency
(spikes/second)
10°
rsu
0
0°
10°rsd
position
of crab
time (one oscillation = 1.79 secs)


220
165
110
55
instantaneous frequency
(sp. sec$^{-1}$)
10°rsd 0
0°
10°rsu
position of
crab
time (I oscillation = 1.79 secs)

6 sp.sec$^{-1}$ in the 20 second duration of the run. This finding supports the hypothesis that the rotational sensitivity is not visually mediated.

There was at least one other unit present in the trace which appeared to respond in the same way as unit XV. It was too small for accurate quantitative analysis.

Summary of the properties of the phasic directional units

| Unit | Tract Recorded from (left or right) | Most Directional Orientation | Phase | Relationship to | Position |
|---|---|---|---|---|---|
| VI | r | 75°/255° | 93° | lead on | hd |
| VII | r | 180°/360° | 73° | " | rsd |
| VIII | r | 135°/315° | 90° | " | lsd/hd |
| IX | - | 60° | 56° | " | hu/rsu |
| X | l | 75° | 82° | " | hu |
| XI | r | 180°/360° | 48° | " | rsd |
| XII | r | 180°/360° | 81° | " | rsu |
| XIII | l | 15°/195° | 9° | " | rsu |
| XIV | l | 120°/300° | 92° | " | hd/lsd |
| XV | l | 180°/360° | 86° | " | rsd |

The underlined orientation is the one to which the phase relationship data applies.

# CHAPTER 4

# DISCUSSION

*INTRODUCTION*

Using the same grouping as in the results section the similarities and differences between the units will be discussed briefly before going on to a more detailed consideration of the pattern of directionality and its possible significance to the animal. Other properties, such as gating and multimodality, are treated in a section dealing with coding of vestibular information. The chapter concludes with suggestions for possible future approaches to the analysis of central nervous processing of vestibular information in crabs.

*THE TONIC UNITS*

The property of space constancy described from decapod visual fibres by Wiersma and various co-workers (best summarised in Wiersma 1975) features a maintained inhibition mediated by the statocyst (Wiersma and Yamaguchi 1967). As this requires a sustained inhibitory input, units with a tonic response to maintained tilt were tested very carefully. Only two were found, and both of these showed slight adaptation.

The two units (I and II) were found together in the one trace and it is possible that an existing tract of tonic units occurs in a position inaccessible to the techniques used here. Units found using pin electrodes (VII, VIII, IX, X and XII) showed differences in some properties, such as resting discharge, from those recorded with broken micro-

pipettes, suggesting a selectivity associated with each technique.

Although recorded at the same site these two units showed their strongest responses to markedly different directions of tilt. Unit I responded most strongly to a maintained position intermediate between head-down and right-side-down, while unit II responded to maintained left-side-down. Their polar plots (Figs. 9 and 12) are also different in form but this could be a function of the angle of tilt at which the plots were made. Unit II, which has a much lower gain over small tilts than unit I, was shown to have a much higher rate of discharge when tilted to 25° in the optimum direction than it did at 15°. If this was accompanied by stronger inhibition in the opposite direction of tilt, then the polar plot at 25° tilt would begin to resemble the plot of unit I at 15°.

The response of unit I to sinusoidal oscillation was interesting in several respects. Although for static tilts it appears to code position with accuracy the peak response to sinusoidal oscillation (0.56 Hz, 20° ptp) is close to the peak of velocity (75° lead on position). Considering the variation between cycles (range 54° to 91°) this value is not greatly different from many of the values of the more phasic units (see table, page 47).

Non-linearities in the deflection/frequency curves of all statocyst hairs cause variations in phase relationships dependent on initial hair position (Janse and Sandeman, in preparation). As the phase relationships of the units were determined in different orientations with different starting positions, constant values cannot be expected.

The mass of the statolith, variable from animal to animal (unpublished observations) will also influence the phase relationships (Guedry 1974).

Although Wiersma has suggested that primary sensory

fibres will be found in the optic tract the excitatory tactile input and spontaneous fluctuations in discharge rate show that unit I is a higher order unit.

By direct stimulation of individual hairs Janse and Sandeman (in preparation) have shown both phasic and phasic-tonic response from statolith hairs. They have also shown that the response alters when the stimulation is delivered by manipulating the statolith. Presumably units I and II have input from the phasic-tonic hairs, but statolith responses to both phasic and tonic stimuli need to be investigated in the intact system.

*THE NON-DIRECTIONAL PHASIC UNITS*

The possible function of units responding to movement in any direction (III, IV, V) is not easy to suggest, nor has it been proven that these are in fact statocyst units. If not, they would have to be from another movement-sensitive system. As the legs have been removed and visual effects either tested for or masked, a visceral input is the only other serious possibility. Visceral input has been shown to be crucial to certain equilibrium reflexes in the pigeon (Biederman-Thorson and Thorson 1973). However the responses observed in these three neurons do not have to be involved in any specific equilibrium system. A general movement sensitivity could well have simply a gating effect on other systems.

If a statocyst input is assumed, the difficulty becomes how to obtain a non-directional response from an organ with unidirectional responses aligned along definite axes. The strong response to rotations in any vertical plane could be achieved by pooling the inputs from all the lower thread hairs in both statocysts. Each canal has two populations of hairs responding to opposite directions of movement. As the two vertical canals are at right angles to each other, any rotation in a vertical plane must excite at least one group

of receptors. The two populations of thread hairs in one horizontal canal would be sufficient to obtain the strong yaw response. Thus it appears quite possible to obtain non-directional responses from canal units. Similar pooling would presumably allow these responses to be obtained from the statolith hairs also.

Further investigation of the statocyst may reveal a different basis for these responses. Vertebrate vestibular afferents are generally regarded as being unidirectional yet units responding to both directions of movement have been reported in the skate (Roberts 1975). The presence of similar primary sensory units in the crab cannot yet be ruled out.

*THE DIRECTIONAL PHASIC UNITS*

Unit VI bears obvious similarities to the preceding units, particularly to Unit V which also has a low basic discharge and responds to movements in both directions with the peak response roughly coinciding with peak velocity. The major difference between these two is that unit V responds equally strongly to movements in both directions in any orientation, whereas unit VI responds equally strongly in both directions in few orientations. It is this systematic variation in the response in *one* direction which is the basis of the directionality indicated in Figure 14. The statolith origin of rotational sensitivity in unit VI is clear and against this background the discovery of indiscriminatory rotation sensitive units is not surprising. As in all statocyst interneuronal studies the function of this unit has yet to be revealed, but here the sensory basis of the directionality is the principal problem.

The remaining units (VII to XV) have one feature in common with unit VI. They all discriminate between directions of movement, this directionality being clearly evident

only in a restricted number of orientations. This is one of the few generalizations which can be made about these units. While most of the units show phase leads on peak position of about 90° (see table, page 47) this cannot be correlated with any other property such as basic discharge or strength of response (number of spikes per cycle). Strong responses to rotation could be elicited from all the units with a basic discharge greater than three spikes per second, but low basic rates did not necessarily mean weak responses to rotation (compare units X and XI). There was no correlation between the excitatory direction of rotation and the laterality of the tract recorded from. As only three successful recordings were made after removal of a statocyst the preferred direction of rotation cannot be correlated with a particular statocyst. As two of the three recordings implied bilateral inputs (units VI and XV) it is unlikely that a strict pattern of excitation by ipsilateral rotation will be found, in contrast to the demonstration of ipsilateral dominance reported in statocyst-mediated crab eye movements (Sandeman and Okajima 1973a).

As the origin of the input is obscure in most cases it is not possible to generalize on the pattern of phase shifts caused by different starting positions. However in most cases the shifts were small and the ranges overlapped considerably.

One reason for this negligible difference may be the length of time (< 10 seconds) which the animals were held in the deflected position before release. Any change in body position with respect to gravity will cause hairs to assume a new equilibrium position. This does not happen immediately in thread hairs, and the times for other hairs are not known. After less than 10 seconds in maintained head-up position of 6.5°, *Scylla* circumoesophageal connective fibre 5 with thread hair input, appears to show a phase shift of 30°-60° from when released from the head-down position (Fraser and Sandeman 1975). Further, when released from the head-down

position it did not fire to head-up rotation in the first cycle although irrigation experiments had shown it sensitive to this direction of movement. None of the inputs described here showed phase changes at all similar to those of fibre 5. In particular all responded on the first half-cycle of rotation in their preferred directions. At the one tested frequency (0.56 Hz) all units studied showed peak responses leading the peak displacement in the direction of response. Frequently the peak response was close to the velocity peak (a 90° lead-on position) as is shown in the table. Even unit I, sensitive to maintained tilt, conformed to this pattern with a 75° lead-on peak position during sinusoidal oscillation. The phase relationships should vary with changes in frequency of oscillation, the pattern of change being different for canal and lith systems.

A phase/frequency analysis of all components of the intact sensory system would be of interest not only from the point of view of sensory physiology but as an essential basis for the understanding of which of the parameters of the sensory input the central nervous system is abstracting.

*THE DIRECTIONALITY OF THE PHASIC UNITS*

The directionalities of the phasic units are summarised in Figure 41, where each of the lines represents an orientation in which one unit was best able to discriminate between the two directions of oscillation. The longer the line the greater the number of units with optimum directionality in this orientation, there being two units in the 75°/255° orientation and four at 180°/360°. No distinction is made between the two possible directions of movement in each orientation. The two units with maxima at 75°/255° responded to opposite directions of movement and were recorded from opposite tracts. The ten phasic directional units between them had maximum responses in six of the available twelve possible different orientations (twelve possible

using 15° intervals) and there is obviously no direction of movement in which *Scylla* would not have a unit responding in a sensitive part of its range.

The other obvious feature of Figure 41 is that a disproportionate number of the units recorded (four out of ten) were found to have optima in the 180°/360° orientation. Three (VIII, XI and XV) responded to counterclockwise rotation and one (XIII) to clockwise rotation.

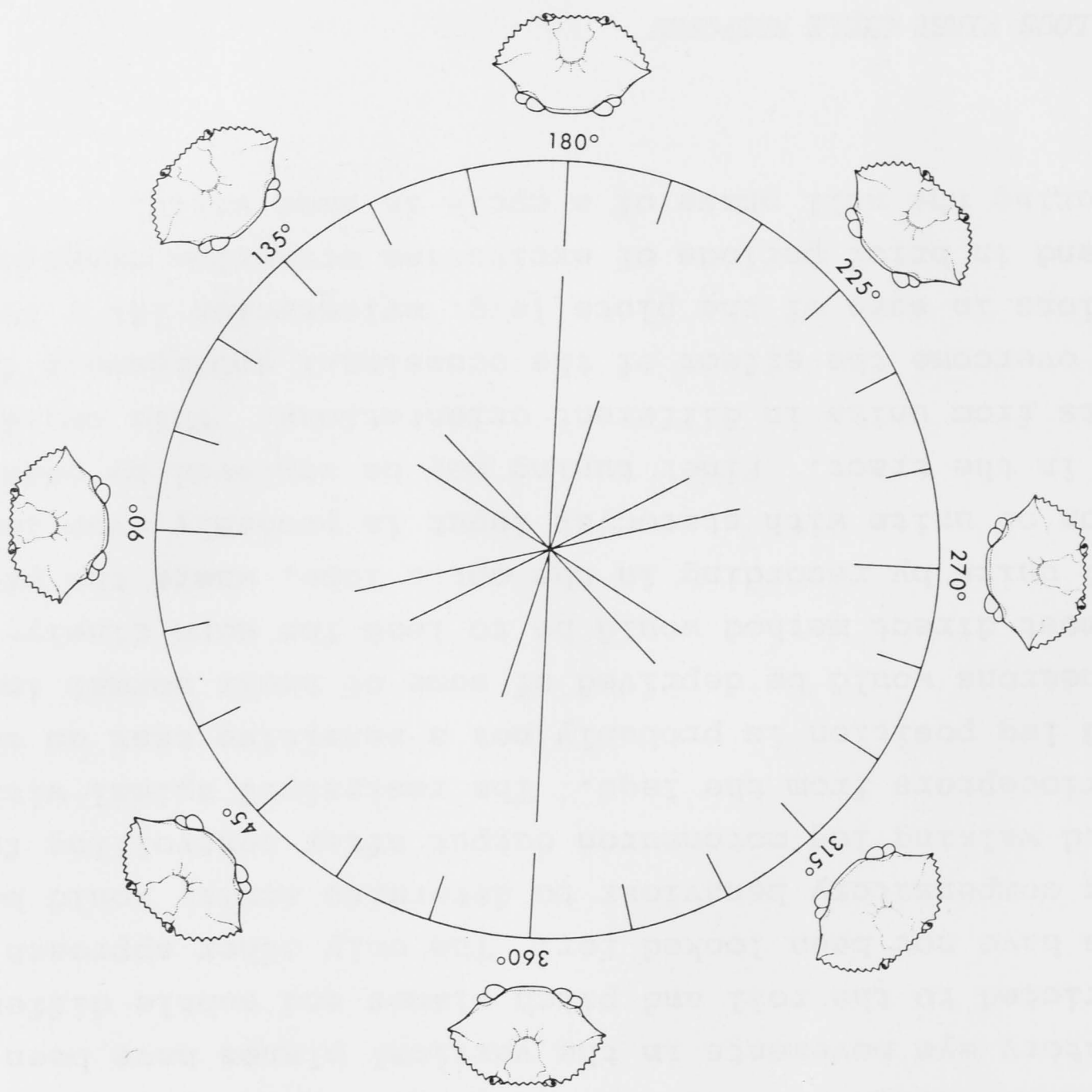
Although Fraser and Sandeman (1975) have shown how canal units might discriminate pitch from roll, a canal unit would always have an optimum response when rotated in the plane of the canal (as is discussed in the results – unit XV). The ability of the canal system to distinguish between pitch and roll depends on inhibition from one group of canal units. As these four "roll" units all have elevated rates of discharge in the orientations showing no directionality (the pitch orientations in these units) and they do not have maximum responses to optimal canal stimulation, there is no evidence that they are canal units.

A larger sample size is necessary before it can definitely be concluded that *Scylla* has more interneurons to roll than to other directions of rotation. However it can be stated with certainty that *Scylla* has phasic, rotation-sensitive statocyst input to the optic lobes, most of which is not derived from the canals.

The pronounced effect that orientation has on the directionality of a response is readily apparent in any of the polar plots, but the shape of the plots reveals a potential for ambiguity in the coding of direction. For instance there appears to be nothing in the response of a single unit to distinguish between movement in the most excitatory orientation and movement in another plane with an increase in stimulus strength to compensate for the suboptimal orientation. Most of the plots are similar in shape, but not in alignment, to the theoretical canal plots (Figures 7 and 8)

*Figure 41*

The orientations giving maximum directionality for each of the phasic directional units.


180°
135°
225°
90°
270°
45°
315°
360°

which are in turn similar in shape to the polar plots of single statocyst hairs (Janse and Sandeman, in preparation). That is, these interneurons are no more finely directionally tuned than the primary sensory neurons. This raises two questions. Firstly, is the crab able to discern the direction of rotation more precisely than at the level of these interneurons, and if so, how? Behavioural studies on compensatory eye movements in the vertical planes have been restricted to the roll and pitch planes and subtle differences have not been looked for. The only other approach using compensatory behaviour to determine acuity would be to record walking leg motoneuron output after controlling for proprioceptors from the legs. The restrained animal with fixed leg position is probably not a sensitive test as the motoneurons would be deprived of some of their normal input. The most direct method would be to look for more finely-tuned units by recording in the optic lobe, where the proportion of units with statocyst input is probably even lower than in the tract. Finer tuning may be achieved by parallel inputs from units in different orientations. This could also overcome the effect of the occasional spontaneous fluctuations in some of the plots (e.g. orientation 180°, Fig. 24) and in brief periods of excitation occurring occasionally during the null phase of a cycle in some units.

*ANOMALOUS FIRST CYCLE RESPONSE*

Certain units, such as unit X (Fig. 27) show responses on the first cycle of oscillation which differ from the general pattern found in the subsequent cycles. Excitatory responses are accentuated and inhibitory responses are diminished during the first half-cycle when the initial direction of movement is excitatory. This gives an apparently greater directionality during the first cycle (Fig. 32). In orientations where the directionality is weak, for example close to the positions of apparent phase change, the excita-

tory effect of the first half-cycle after pendulum release may even be stronger than the second half despite the reversal of this dominance in succeeding oscillations. Although only units which did not habituate, or habituated only slowly, have been studied in detail it should be noted that most preparations revealed units which fired upon the release of the pendulum but which had adapted or habituated completely by the second or third oscillation. These units were ignored.

While Figure 32 shows that even the first cycle may reveal similar directionality to a plot averaged over several cycles, the irregularity of the initial response requires consideration, as a crab can not use the temporal averaging available to the investigator. The first cycle response is probably an anomaly introduced by the experimental procedure and can be thought of as a response to the initiation of movement, where gating effects briefly raise the excitability of the unit. In the walking or swimming crab this phenomenon is probably never revealed as the continuous minor oscillations caused by locomotion would allow adaptation of such a startle response.

### *POSSIBLE FUNCTIONS OF VESTIBULAR INPUT TO THE OPTIC LOBE*

The existence of vestibular input to the optic lobes raises several questions the first being whether or not it is integrated with visual stimuli. In the case of units VIII and XV, both of which are apparently gated by visual input, one form of integration is clear. This does not give any clue to the function of the vestibular unit. In a series of papers Wiersma and various coworkers have described what they call "space-constant fibres" which show inhibition of a part of a visual field by statocyst input. This was always dependent on maintained position, and while it is not impossible that a similar class of fibres is

inhibited by phasic units of the types found in this study, only units I and II might possibly be involved in the type of interaction Wiersma has described.

Another possibility is that these units are part of a system allowing discrimination between movements in the visual field caused by the animal's bodily movements and those caused by actual displacement of an object in the visual field. This proposal differs from the space-constant system in that both the inhibitory (statocyst) and excitatory (visual) fibres are stimulated by movement, whereas in the space-constant fibres it is the size of the field which is limited, not the response. It is a similar concept to the efference copy neurone described by Johnstone and Mark (1971) but their system involved a motor system corollary discharge compensating for the self-stimulation which results from voluntary movement.

This form of inter-sensory efference would be similar to the purely visual inhibition of the LGMD fibre by whole field movements in locusts (Palka, 1969, O'Shea and Rowell 1969). Here the response to restricted field movement is largely inhibited during whole field stimulation. Presumably these units are not able to detect the movement of an object when the animal is in motion. The suggested vestibulo-visual system of discrimination between movements is not, then, a complex construct to explain a function which the visual system alone can accomplish. The spatial constancy demonstrated in bees by Wehner (1975) is purely visually mediated, whereas the space constant fibres in decapods (Wiersma and Yamaguchi 1967) although less elegantly analysed, appear to have a statocyst input. The phenomena in the two groups are not entirely equivalent.

The crab has been shown in behavioural experiments (Horridge and Burrows 1968) to possess a different type of movement detecting system from that described in insects above. *Carcinus* detected oscillations of a striped drum

during voluntary eye movements (which produce whole field stimulation) which did not modulate the optokinetic response of the blinded driven eye. This was achieved without any efferent information on the movement of the seeing eye. A non-visual reference is implied. Statocyst input, already shown to evoke eye movements, could gate the response to the whole field movement caused by eye withdrawal.

Although rotation of a crab has been shown to evoke compensatory eye movements (Dijkgraaf 1955) the gain of these is less than unity (Silvey and Sandeman 1976a). Therefore a rotation of the body will still present rotational information. During swimming substrate contact is lost, greatly reducing the ability to deduce the position of the body by limb proprioception. This leaves only the visual and vestibular systems to provide references against which movement can be detected. *Scylla* often lives in turbid water and is active at night (Stephenson, pers. comm.), conditions where there is little background texture against which the relativity of movements might be discerned. This may be achieved visually using the pattern of light polarisation existing in water containing suspended particles (Waterman 1975). It has long been known from behavioural experiments that *Ocypode* can detect changes in the e-vector of plane polarised light (Schöne and Schöne 1961). Recently Leggett (1976) recorded from interneurons in *Scylla* showing uni-directional responses to rotation of the plane of polarisation. As well as the suggested role in maintaining stability while swimming, these could well be part of a system providing the visual cues necessary for the unambiguous detection of movements of other objects.

Vestibular efferents to the optic lobe might equally well provide this reference system. Ideally they would have to be in phase with position, a situation approached only by unit XIII in this study.

The phase relationship is dependent on the frequency

and angular velocity of the oscillation, as well as the mass of the statolith and it may be that at other frequencies other units will be in phase with position. Note that during continuous oscillation the static position coding unit I shows a peak response leading the position by 85°. That is, a tonic position coding unit does not code position accurately when oscillated at some frequencies.

Our knowledge of both the visual and vestibular systems, the ease of quantification of both their inputs, the accessibility of the two parts of the system to manipulation and the demonstrated lack of eyecup muscle efferents to the optic lobe make the crab decapod system uniquely suitable for physiological analysis of the interaction of the visual and vestibular system of movement detection.

*MULTIMODALITY AND GATING*

Many of the units in this study respond to two or more modalities of stimulus. Most commonly they responded to tactile input to large areas of the carapace units and usually the leg stumps as well. When tactile and rotational stimuli were delivered simultaneously to unit VIII the directionality of the unit was increased. Tactile inputs may have a generally excitatory effect, raising the basic discharge level and thereby allowing more accurate coding of a rotation as discussed shortly. It is possible that in the swimming crab some of the carapace receptors are stimulated by currents, but apart from this no other benefit from combining independently excitatory tactile and rotationary inputs was suggested by any of the units in this study.

In contrast it was clearly obvious in units VIII and XV that visual input gated the rotational response, with much stronger responses being observed where obvious visual cues were presented. Unit XV gave no response during rotation when the statocysts were removed but the same visual cues

presented. Unit VIII was not tested in this way. In neither case did the addition of strong visual input cause a shift in the phase of the response, whereas addition of visual input to statocyst-mediated compensatory eye movements in crabs alters the phase of the response as the visual input completely over-rides the vestibular component (Janse and Sandeman, in preparation). Visually mediated eye movements are always in exact anti-phase to body movements. The failure of visual input to change the phase of units also suggests that visual input is a gating stimulus rather than an additional movement detector and the two inputs do not appear to summate in the manner described from mice (Dräger and Hubel 1975). However the visual input did not simply cause a general increase in the discharge rate and directionality was enhanced not diminished. The nature of the gating response, whether whole field movement, increased light intensity or some other parameter, awaits investigations.

*THE CODING OF ROTATION*

In several directional units the mean firing rate during movement was much higher than the basic level when stationary, even during oscillations at right angles to the most directionally-sensitive orientation. For example unit XIV has a basic discharge of 6 $sp.sec^{-1}$ rising to 20 $sp.sec^{-1}$ during an oscillation in which no constant phase relationship was evident. This has important consequences for the information-carrying capacity of the neuron. The higher the carrier frequency the higher the frequency of modulation (in this case the oscillation frequency) the system will code coherently (Stein *et al.* 1972) and the more accurately will it code lower frequency oscillations. Under the conditions of these experiments (0.56 Hz, 20° ptp) some units showed an approximately sinusoidal modulation of the basic discharge in all orientations (Fig. 37). Others

showed rectifying responses (where the discharge reached zero in the inhibitory direction) in the orientations showing greatest directionality (Figs. 39 and 40). Except for unit VIII all units showed non-rectifying modulation in less directionally-sensitive orientations.

All statocyst receptors so far described from *Scylla* have been shown to be unidirectional (Janse and Sandeman, in preparation). This appears sufficient to explain the modulation of all units recorded here, whether silent or with a resting discharge, but it does not explain the maintained elevated discharge in orientations showing no directionality. The elevated discharge and the directionality may be the products of different inputs. This may not necessarily be so, as mammalian otolith units respond to orthogonal shear (Fernandez and Goldberg 1976b) and certain plots of statolith hairs suggest a similar situation may apply in *Scylla* (Janse and Sandeman, in preparation). Where rectification is present there must be either active inhibition or a situation where the output of the statocyst hairs is entirely responsible for maintaining the resting discharge, complete inhibition of the primary unit causing complete inhibition of the interneuron. Against the latter interpretation is the observed basic discharge in unit XV (which shows strong rectification, Fig. 39) after both statocysts were removed.

Active inhibition was demonstrated in the only unit appropriately tested. Unit VIII showed an excitatory response in tactile stimulation of the carapace. When delivered during oscillation it intensified the excitatory response to rotation, but complete inhibition still occurred during movement in the opposite direction. That is, the excitatory effect of tactile input was negated, indicating an actively inhibitory process.

Active inhibition in the opposite direction to excitation is one mechanism by which the gain of a system can be increased if the system has a high basic discharge. Many

units were observed to raise their mean firing frequencies even during small oscillations in orientations giving non-directional responses. One function of the gating and multimodality, then, might be to raise the firing level to allow more accurate coding. This suggestion is no more than an hypothesis consistent with the recorded data. It is testable by controlling or eliminating various inputs. It is possible to provide very diverse visual stimuli including variations in intensity and in the rate and direction of moving objects in the visual field. Broad or restricted field tactile stimuli can be administered at varying intensities or eliminated completely by selective nerve cutting techniques.

There is some evidence suggesting that the sensitivity of many units may depend on complex interactions, a situation which would complicate interpretation of these properties. When one antennule base was removed both unit VI and unit XV gave much weaker responses to movement, although directional responses were still obtained in both cases. The basic discharge was lowered slightly in unit XV. The basic rate before ablation was too low (0.5 $sp.sec^{-1}$) in unit VI for any change resulting from the ablation to be apparent. Although visual input altered the response of unit XV to rotation the basic discharge was not changed and purely visual input was insufficient to cause a response without associated statocyst input. It therefore appears that in both these units the detection of rotation involves a complex system of interaction involving both statocysts. The most important question is whether both statocysts contribute to directionality or does one simply help maintain the sensitivity of the system. The second possibility may well have been the case for unit XV as the gating effect of visual input in the intact animal was much weaker in the intact animal than in the unilaterally extirpated animal, where the sensitivity had been reduced. In addition the phase relationships were observed before and after removal of one statocyst were similar.

The only other successful recording after antennule base removal was of unit XIV which showed no alteration in either its basic discharge or its response to movement.

*CONCLUSION*

A profitable approach to the analysis of vestibular interneurons would be to test each unit with a wide range of frequencies and amplitudes of sinusoidal oscillation on different orientations, and also about mean positions other than the horizontal. This would reveal the extent to which the apparent directionality is dependent on the starting position of the hairs. These show a sigmoidal curve when the frequency of response is plotted against hair deflection (Janse and Sandeman, in preparation). As explained earlier (see Techniques – "The orientation of the statocyst canal") only a receptor responding to increasing deflection in a purely linear manner will give a circular plot. If a hair is in the non-linear part of its range the response will not vary linearly with the hair deflection. In such a situation a circular plot would not be obtained. Therefore testing the directionality during oscillations about several mean positions is necessary to prevent spurious impressions of the angular sensitivity of any unit. It would also show the capability of the system to deal with a range of frequencies and magnitudes of movement without distortions in gain or phase.

In accordance with this it is necessary to know the response of the primary receptors to the same range of stimuli. Without this the central processing of statocyst input cannot be adequately treated. In addition the four different known sensory hair systems of the statocyst (inner and outer lith hairs, free hook hairs and thread hairs) can be expected to show different input and output relations. Knowledge of these may allow the localisation of the sensory input of an interneuron. Otherwise this vital information

must be obtained either by selective sensory nerve cutting or, less ambiguously by direct hair stimulation in the opened statocyst. Several of the units in this study indicate a complex array of interactions determines their output. The apprcach just described may simply confirm this complexity and also the great diversity already demonstrated in directionality and other properties.

While the central nervous coding of vestibular information on crustacea awaits thorough examination, this study raises a more important question. Phasic statolith and possibly canal input has been demonstrated in the optic tract, where it does not lead directly to any motoneurons. What then is its function?

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